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PRINCIPAL INVESTIGATOR: Thomas E. Wiese

CONTRACTING ORGANIZATION: Xavier University of Louisiana
New Orleans, LA 70125

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14. ABSTRACT
Xavier University (XU) and the Tulane Cancer Center (TCC) will build a core of human talent that will address scientific problems such as drug resistance and the effect of environmental agents on breast cancer (BC) in the African-American community. A multi-part research and training program will generate data, develop new research programs and train new faculty and African-American students in BC research. The first component will fund two research projects. The Wang and Burow project will elucidate a previously unexplored cellular signaling mechanism that leads to drug resistance in breast carcinoma cells derived from African American women and women of other ethnicities. The Wiese and Hill project will identify and characterize the genes and gene products associated with BC cell proliferation induced by exposure to pesticide mixtures and is relevant to the African American community in Southern States where pesticide exposure is relatively high. The second part of the program aims to increase the number of faculty at XU involved in BC research by supporting two junior faculty members to develop BC research projects with a TCC mentor. The third objective will support research training of XU undergraduates and pharmacy students. The fourth objective will provide workshops, seminars and research opportunities in BC research for the XU community. This program will enhance the understanding of unique aspects of BC development and progression among African American women and will contribute to the elimination of the "mortality gap" between African-American BC patients and women of other ethnicities.

15. SUBJECT TERMS
MCF-7, MEK5, Environmental Estrogens, pesticides, Proteomics

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Introduction

African American women are at higher risk for breast cancer (BC) mortality compared with their white counterparts. Over the past decade BC mortality has decreased 1%-2% per year in white women, but not in African-American women. The resulting "mortality gap" is a serious national problem, and understanding the reasons for it and developing solutions must be a high priority. Thus, BC research must focus on developing breast cancer models that would aim to accurately predict the disease development and progression among African-American women. ***We are convinced that increasing the involvement of African American students in BC research will greatly contribute to increasing awareness of the disease in the African American community, which in turn will increase the likelihood of early detection of the disease. Furthermore, the focus on the unique aspects of BC in African American women will lead to better understanding of the disease, and to better treatment options for African American women. This will eventually minimize or eliminate the BC "mortality gap".*** To this end we are developing a training program at Xavier University of Louisiana (XU) in collaboration with the Tulane University Cancer Center (TCC). More than 90% of Xavier's student body is African American has active programs (MBRS, MARC, RISE, NSF/MIE) designed to increase the number of minority students pursuing careers in medical and biomedical research. Through this BC training program, African American students will have the opportunity to become involved in BC research. Tulane (TU) and Xavier have a long history of collaborations involving joint centers and programs and individual collaborations between Tulane and Xavier faculty and staff are common. This new initiative will provide funds for yet another collaboration offering a unique opportunity for XU researchers to establish a BC research program for the benefit of XU students and, eventually, the African-American community. The goals of the training program are to create an environment that fosters BC research, in which XU investigators will receive substantive training and to complete substantive research projects of high relevance to the eradication of BC. The program will enable XU investigators to publish their results in peer-reviewed literature and advance toward independently funded BC research programs. The program includes two full research projects that involve an XU researcher and a qualified TCC mentor. The program will identify two additional XU researchers who have expressed an interest in BC research but do not have prior funding in BC. Participating XU faculty will get the opportunity to network and learn about BC research through participation in the TCC weekly seminar program and the signal transduction workshop that will focus on breast and prostate cancer. The two additional XU faculty involved will develop a mini-proposal in Y1-2 and carry out pilot studies with the advisory of a mentor faculty from TCC in Y2-4. The results of all program research studies will be used as a basis for future proposals in the area of BC. Yearly symposia will be held to provide information to XU students and faculty as well as to enrich the experience of the participating members regarding research opportunities in BC. Multiple project group meetings will be held each year to discuss current data, manuscripts in preparation, funding opportunities and issues regarding project operations.

Year Three Progress

The most significant factor effecting progress in Y3 of this program was the continuing effects of Hurricane Katrina. Xavier University of Louisiana did an amazing recovery from the storm and after all buildings on campus damaged by flood waters, reopened in January 2006. While no lab facilities involved in this program were directly impacted by flooding, all perishable supplies were lost. I addition, faculty involved in the program have been burdened with an increased teaching load after the evacuation and to make up for the lost time in the fall semester 2005, Xavier continued the fall in Jan-April 2005 and plans to run the Spring semester 2006 in the summer of 2006. Thus, time for research activities has been reduced in the programs Y2 and Y3. For more detials on the impact of Katrina on Xavier and this program as well as the program

status in April 2006, see the April 2006 DOD BC reverse site visit presentation from Xavier in Appendix 1 below. All teaching loads and schedules did return to normal in Fall 2006. To make up for lost time and to save this program, we asked the DOD for a one year funded extension (Y5) in May of 2006 that was subsequently approved (see Appendix 2).

During the Katrina evacuation, the Tulane Cancer Center and the Louisiana Cancer Research Consortium (LCRC), our partner in this DOD program offered $25,000 grants to re-supply cancer research labs impacted by Katrina. This DOD Xavier program was allowed to compete for one of these Tulane/LCRC grants, the PI Dr. Wiese submitted a proposal while evacuated, it was awarded in November 2005 and these funds have been used since January 2006 to re-supply the labs of Dr. Wiese and Wang at Xavier so that this program can recover. See Appendix 1 in the Y2 progress report for more information on this funded recovery proposal.

The main goal of this program in Y3 was to re-establish our laboratories and then re-establish our research projects. Considering the heavy teaching loads from Jan-August 2006, the only research activities we were able to accomplish for that period involved getting labs and research back in operation. The fact that the summer period, which is typically a time to focus on research, was not available has made a large impact on the program. With both of the main pilot projects involving cell culture, this meant breaking out cells and tests to make sure the cells behaved the same now as they did in experiments before Katrina. In addition, considerable amount of time has been spent in both pilots repeating key research findings from the previous years of the program. With project aims built on each other in a sequential way, it is critical that we know that we can repeat all findings with the new reagents and new batch of cells. With the help of the recovery grant from the Tulane Cancer Center and LCRC, we have been able to replace many of the lost items in our labs. However, with university downsizing and turnover to staff in our fiscal office, purchasing has been slower than normal. We have also incurred some delays because our Tulane collaborators are also trying to recover their labs while being faced with more teaching duties. Another challenge to the program and also have more teaching and other duties. Dr. Hill at Tulane is now chair of his department. Dr. Wiese is now PI of both the Xavier DOD Breast Cancer and the Prostate Cancer programs as well as manager of the new NCI P20 grant at Xavier.

It is important to note that by early 2007, the projects in this program (Wiese-Hill, Wang-Burow, Wolfgang-Miller) each had their research labs fully operational and research productivity back to at least where they were prior to Katrina. The personal and administrative task of bringing back these labs and maintaining these teams should not be underestimated. The combination of intense personal efforts by all involved from Xavier and Tulane combined with the immediate aid of the Katrina recovery grant from Tulane Cancer Center (LCRC) and the award of a funded extra year by the DOD has brought this program back together in a way that program aims can be accomplished: Xavier will develop self sustaining cancer research programs well into the future.

Body

<u>Task 1</u>
Complete two substantive research projects of high relevance to eradication of breast cancer

Project 1
Chemoresistance in Breast Carcinoma Cells: MEK5-BMK/Erk5 Expression and Proteomic Analyses"

Guangdi Wang, Ph.D., Department of Chemistry, Xavier University of Louisiana PI (Trainee)
Mathew E. Burow, Ph.D., Department of Medicine, Tulane University School of Medicine (Mentor)

Aim 1: To demonstrate the requirement for and the role of the MEK5 pathway in survival signaling and suppression of apoptosis in MCF-7 breast carcinoma cells.

(1). Implicate MEK5 activation in cell survival signaling, prevention of anti-estrogen and chemotherapeutic drug-induced cell death using MCF-7 stable, transiently transfected cells and ZR-75-30. (Months 1-18).
(2). Implicate apoptotic suppression as a mechanism for MEK5-mediated survival and drug-resistance (Months 12-24).

Year Three Progress

One feature observed in the differences between the MCF-7VEC parental and MCF7-MEK5 cells was an observable epithelial to mesenchymal transition (EMT) (Figure 8). This suggested a focus for novel effectors of MEK5 as targets in the regulation of EMT. To further explore this RT-PCR analysis of gene involved in EMT were analyzed in the MCF7-(VEC), MCF-7(MEK5) and MCF-7(MEK5)-(Erk5-shRNA) cell lines (Figure 9). We observe a noticeable loss of E-Cadherin and beta-catenin in the MCF-7(MEK5) cells. Expression of both beta-catenin and E-cadherin are restored with Erk5 knockdown in these cells. Expressin of Slug and Zeb1 (ΔEF1) are increased in the MEk5 cells. These data suggest that the MEk5 overexpression promotes and EMT like phenotype in breast carcinoma cells. These data suggest that Slug expression may be an immediate downstream target of MEK5-Erk5 signaling. We expect the EMT gene expression differences to be confirmed by proteomic analysis of the THE cell lines as well as through western blot. Future direction will explore the ability to selectively knock down Slug expression using shRNA strategies.

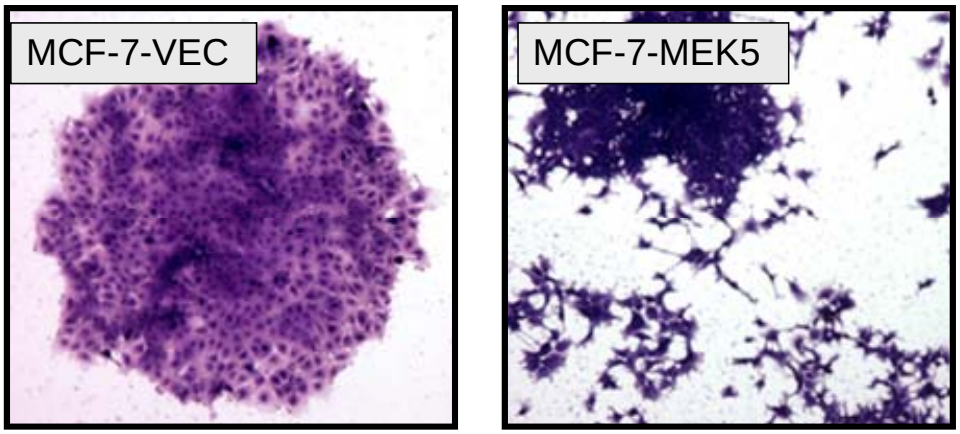


Figure 1. Stable MEK5-Erk5 activation promotes epithelial to mesenchymal transition.. MCF-7(VEC), and MCF-7(CA-MEK5) cells were plated in 6 well plate (100 cells/well) in 10%-serum containing media. After 10 days in culture cells were harvested and colonies stained with crystal violet. Clear morphologic differences in the MCF-7(VEC) and MCF-7(CA-MEK5) cells can be observed.

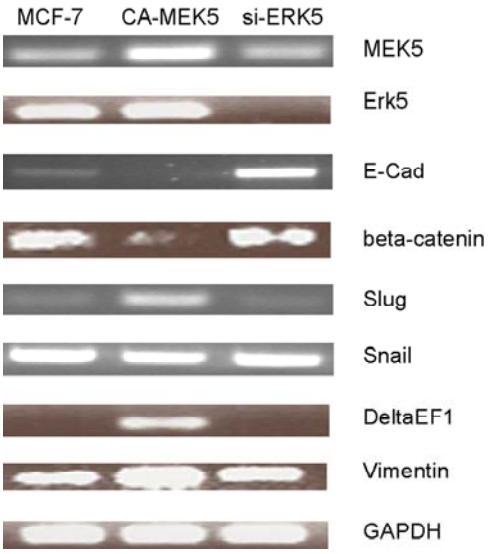


Figure 2. Stable MEK5-Erk5 activation regulates gene expression associate with EMT. MCF7-VEC, MCF-7(CA-MEK5)-(shRNA-empty) and MCF-7(CA-MEK5)-(shRNA-Erk5) cells were grown in 10%-serum containing media and harvested after 72 hours. RNA isolation and RT-PCR analysis of MEK5, Erk5, E-cadherin, β-catenin, Slug, Snail, ΔEF1 (ZEB1), and Vimentin was performed Data shown is representative analysis of three independent experiments.

Analysis of proteins potentially involved in difference sin the MEK5 and VEC cels suggested a role for metalloproteinases and Cox2. Using Western blot analysis we demonstrate that MEK5 cells exhibit an increased expression of Cox-2, MMP-9 and phospho-PKCalpha. These targets will be pursued as potential downstream efforts of the MEk5 phenotype in breast carcinoma cells.

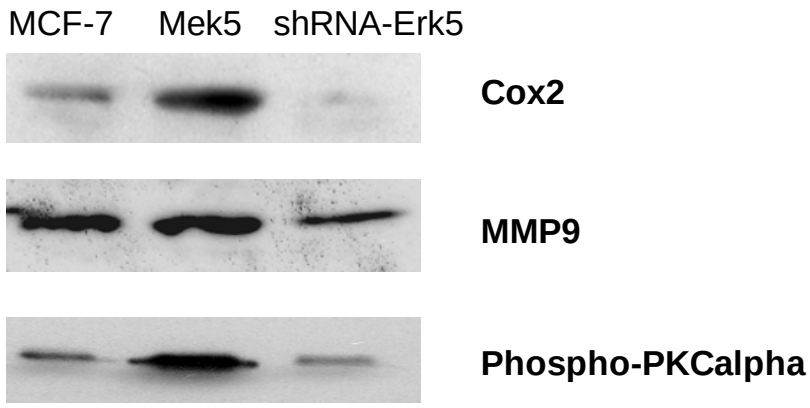


Figure 3. Stable MEK5-Erk5 activation regulates gene expression associate with EMT. MCF7-VEC, MCF-7(CA-MEK5)-(shRNA-empty) and MCF-7(CA-MEK5)-(shRNA-Erk5) cells were grown in 5%-CS-serum containing media and harvested after 72 hours in estrogen free media. Western blot analysis of Cox2, MMP-9 and phospho-PKCα expression was analyzed. Data shown is representative analysis of three independent experiments.

Aim 2: To characterize differences in protein expression between MCF-7N (APOP-Sensitive), MCF-7M (APOP-Resistant) and ZR-75-30 breast carcinoma cells and identify anti-apoptotic proteins, such as Survivin, within MEK5-expressing cell lines.

(1) Prepare samples for 2D gel separation. (Months 18-24).
(2) Separate proteins on 2D gel electrophoresis, compare differences in protein expression, and perform in-gel tryptic digestion of excised protein products. (Months 24-36).
(3) Sequences obtained from tryptic digests will be used to characterize and identify protein expression differences between drug resistant ZR-75-30 and MCF-7 breast carcinoma cells with a focus on known anti-apoptotic proteins or novel apoptotic domain containing proteins (BCl-2 homology (BH), baculovirus IAP repeat (BIR0, caspase activation recruitment domain (CARD), etc.). (Months 24-36).

Year Three Progress

Overview
Collaboration continued smoothly between Dr. Burow and Dr. Wang in their joint efforts to achieve the proposed research objectives. The major progress made in the past project year can be summarized as follows:

1. Instrument Acquisition: An HPLC-tandem mass spectrometer tailored for proteomics work was purchased in October 2006; installation and on-site training were completed in December of 2006. The instrument is now the workhorse of our proteomics analysis experiments. We also purchased a gel spot cutter and a digestion station for protein sample preparation and treatment prior to mass spectrometric characterization. We now have a complete line of equipment for mass spectrometry based proteomics work.
2. Hiring of a new post-doc who has working experience in MCF-7 cells and formal training in molecular immunology. Mr. Changhua Zhou is finishing his Ph.D. in immunology in Sichuan University in China and has joined our lab since April 1, 2007.
3. Our new mass spectrometer has allowed us to acquire more results than previously possible. Gel and protein results are presented in the next section.

Results and Discussion: We have now established protocols to carry out cell culture work in our own lab (Figure 4). This has greatly improved our research productivity in obtaining sufficient amount of proteins for each cell line to do replicate gel analysis. To ensure reproducibility of 2D gel results, cell lysates are pooled and protein concentrations determined before loading on the IEP strips. Work is underway to identify all differentially expressed protein spots shown on 2D gels and to conduct RT-PCR and Western blot analysis for confirmation of selected proteins that are found up-regulated or down-regulated by over 2-fold (Figure 5).

Gel image analyses using the updated version of PD Quest 8.01 software (BioRad, CA) indicate significantly differential protein expressions between the two cell lines as shown in the scatter plots in Figure 6.

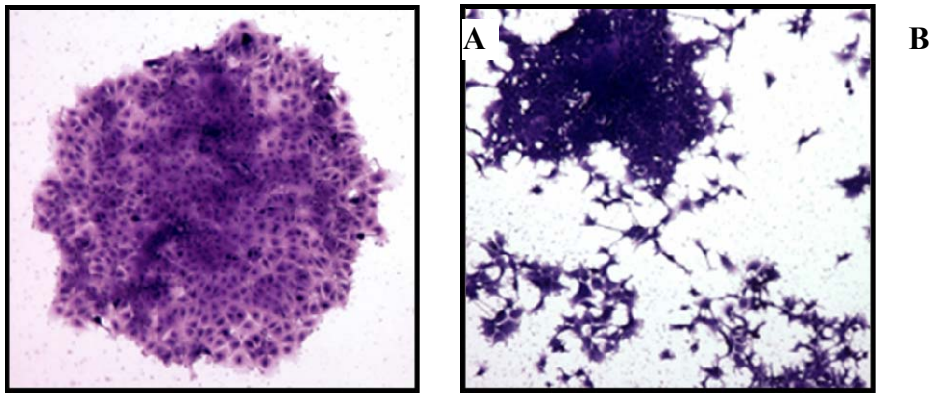


Figure 4. Morphology of MCF-7N (A) and MCF-7MEK5 (B).

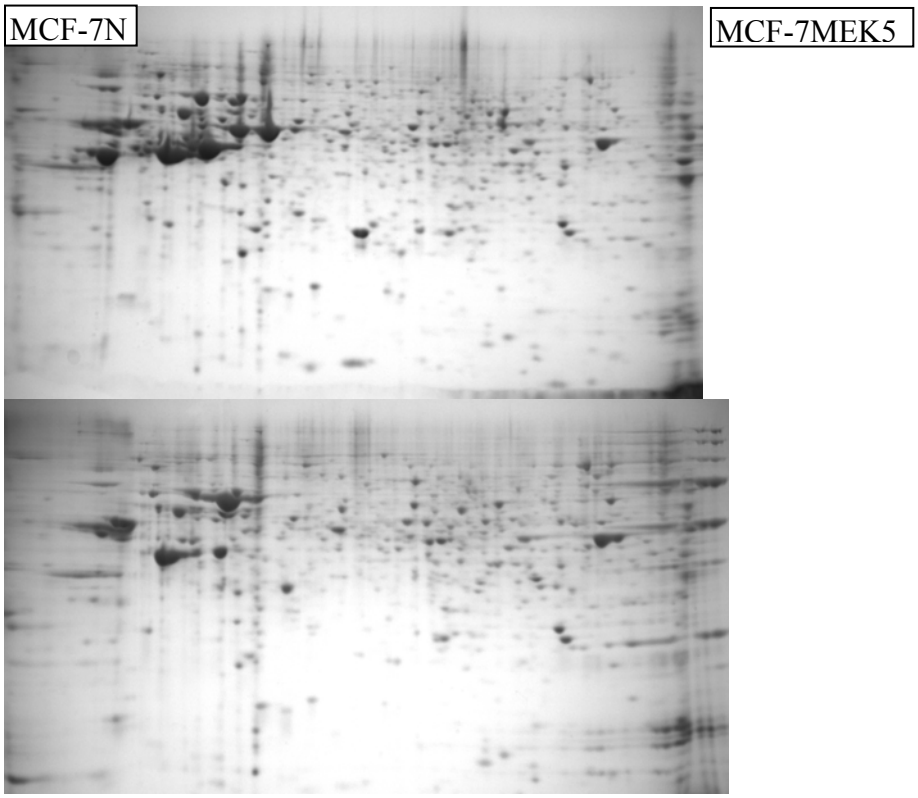


Figure 5. 2D gel images of whole cell lysates of (left) MCF-7Mek5 cells and (right) MCF-7N cells using pH 5~8 IPG strips and 11 cm nonlinear gradient SDS PAGE gel, and coomassie stain.

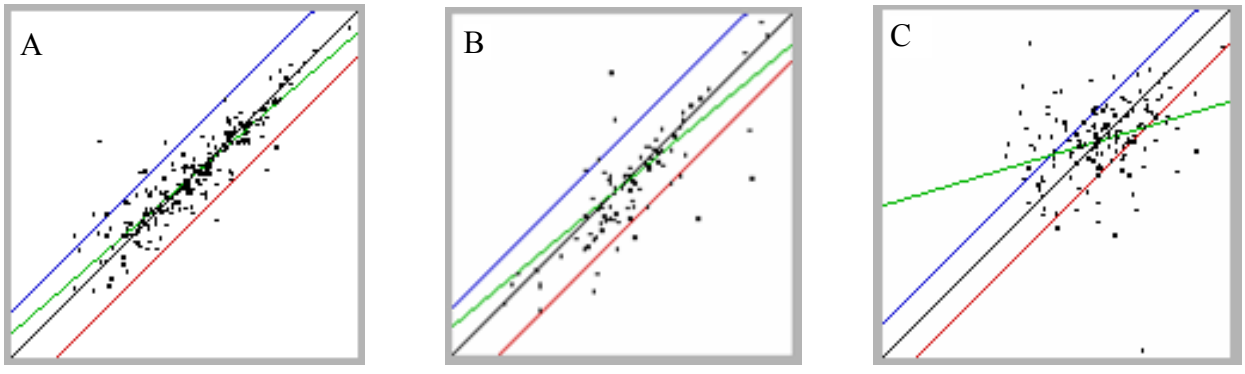


Figure 6. Correlation between (A) two gels of MCF-7Mek5 cell, r = 0.8889, (B) two gels of MCF-7N cell lysate, r=0.7461, and (C) MCF-7Mek5 and MCF-7N cell lysates, r=0.3005.

Gel image analyses using the updated version of PD Quest 8.01 software (BioRad, CA) indicate significantly differential protein expressions between the two cell lines as shown in the scatter plots in Figure 3. Detailed quantitative gel results are summarized in Table 1. Of the 354 quantifiable gel spots found for the two cell lines, 72 protein spots were only expressed in the drug sensitive MCF-7N cells, while as many as 150 were expressed only in the drug resistant MCF-7Mek5 cells. In addition, 19 protein spots were found to be over expressed in MCF-7Mek5 cells by a factor of 2 or greater, and 21 protein spots were over expressed in MCF-7N cells by a factor of 2 or greater. We expect to identify most, if not all, of the over 200 gel spots that could provide valuable information on the signaling pathways that are responsible for the development of drug resistance in MCF-7 breast cancer cells.

Table 1. Summary of Gel Spots for MCF-7N and MCF-7Mek5 cell lysates

Total gel spots that can be characterized by mass spectrometry	354
Number of proteins that are expressed in MCF-7N but not in MCF-7Mek5	72
Number of proteins that are expressed in MCF-7Mek5 but not in MCF-7N	150
Number of proteins that are **over** expressed in MCF-7Mek5 by a factor of >2	19
Number of proteins that are **under** expressed in MCF-7Mek5 by a factor of >2	21
All other proteins spots	92

Table 2. Proteins Expressed in MCF-7MEK5 but not in MCF-7NULL (in progress)

Spot No	MW		pI		Protein Identified
	Theory	Expt	Theory	Expt	
A7	30018	28000	5.15	5.6	Catechol-O-methyltransferase, isoform CRA_b
A8	29537.9	32000	6.16	7.2	Proteasome alpha 1 subunit
A9	47140	49000	7.16	7.2	Enolase A
A12	62600.4	75000	6.39	6.8	Unnamed protein
B1	56625	60000	6.29	6.7	HNRPH1
B3	12847	13000	5.1	5.4	Tubulin -specific chaperone a
B4	16532	15000	5.6	5.3	alpha-CP1
B5	16433.3	17000	4.92	5.3	Hypothetical protein
B7	12252	11000	5.56	5.6	Chain A, Solution Structure Of Mouse Er [Monodelphis domestica].
B8	21512.7	25000	5.22	5.4	Glyoxalase I variant
B10	21512.7	25000	5.22	5.4	Glyoxalase I variant
B11	21512.7	24000	5.22	5.1	Tumor protein, translationally-controlled 1, isoform CRA_c
C03	49199.4	50000	5.86	6.7	Hnrph1 protein
C04	36904	36900	6.4	6.9	Ribonucleoprotein isoform 2
C05	36904.1	36000	6.4	7.3	HNRPH3 protein

Aim 3: In this task we will use RNA interference strategies to validate a role for the Erk5 pathway in downstream gene expression and in suppression of chemotherapeutic drug-induced apoptosis. Our preliminary analysis revealed survivin expression was increased in drug-

resistance and MEK5 expressing breast carcinoma cells. Subsequently we will characterize the role of these downstream targets such as Survivin, in suppression of apoptosis and drug-resistance.

(1) Optimize pSUPER base RNA interference (RNAi) suppression of ERK5 expression in breast carcinoma cells (Month 15-18).
(2) Confirm a role for Erk5 signaling in MCF-7N-CA-MEK5, and MCF-7M-(RESIST) cell survival using pSUPER-Erk5-RNAi. (Months 18-28).
(3) Develop/validate RNAi strategies for Survivin suppression using pSUPER method as above. Use RNAi to implicate Survivin expression in drug resistance and apoptotic signaling of MCF-7 and ZR-75 breast carcinoma cells (Months 24-36).
(4) Develop, validate and use RNAi strategies for novel targets identified from proteomic analysis of drug resistant breast carcinoma cells from Aim 2. (Months 36-48).

Year Three Progress

No New Progress on this Aim in Y3.

Project 2
Interactions of estrogen and progestin active environmental chemicals on BC cell proliferation, survival and gene expression

Thomas E. Wiese, Xavier University College of Pharmacy PI (Trainee)
Steven R. Hill, Tulane University School of Medicine (Mentor).

Aim 1: Examine the effects of binary mixtures of estrogen and progestin active environmental compounds on cell proliferation and survival.
(a). Develop treatment mixture matrix and plan for proliferation experiments (Months 1−2).
(b). Perform cell proliferation studies with binary mixtures of pesticides (Months 1−18
(c). Identify mixtures with novel effects on cell proliferation (Months 6−20).

Year Three Progress
Research Assistant
Mr. H. Chris Segar continues ar research assistant on this project. Primary acomplishments in Y3 involved re-establishing the Wiese lab, repeating proliferation experiments done in this project prior to Katrina to validate that the new cells and new lab reagents worked properly and planning for the genomic stage of this project.

Collaboration between Dr. Wiese at Tulane Cancer Center and Dr. Hill
Dr. Wiese has been in close contact with Dr. Hill since the start of this project through phone, email or meetings. While the project was designed to take place entirely in the Wiese lab, Dr. Hill contimues to provide input on experimental design and data interpretation. The main contribution of Dr. Hill to this project has been discussions relating to the use of microarray technology to identify specific genes or classes of genes that may be related to the observed mixture effects (see Y2 progress of Aim 2 below). Dr. Hill has also provided insight regarding the management of the overall training program (see tasks 2 and 3 below).

Preliminary and Y1-Y2 results summary

The series of pesticides included in this study included isomers and metabolites of DDT and methoxychlor. Each are known to have weak estrogen, androgen and/or progesterone activity. A series of MCF-7 proliferation studies were conducted to identify novel interaction effects of binary mixtures of these compounds. The initial studies were designed to include one pesticide at the lowest observed effect level (LOEL) and the other at the highest dose possible (10-5 M). Experiments were also conducted to determine if mixing the pesticide (high dose) with sub-optimal concentrations of estradiol-17β (E2) enhanced estrogen induced proliferation. This series of experiments did not identify mixture combinations with more than the additive cell proliferation activity expected from the compounds alone at the same concentrations. These same mixtures were examined in the MVLN estrogen responsive reporter gene assay where similar additive effects were also observed. See Y1 progress report for more information. At this point, Dr. Wiese decided to examine mixtures that contained one of the organochlorine pesticides along with one of three organophosphate pesticides. We have observed a positive sensitizing or potentiation effect of organophosphate pesticides on the weak estrogen dependant proliferation activity of organochlorine pesticides (increased potency). This action can be eliminated by antiestrogen and is likely estrogen receptor (ER) dependant. The observation that this sensitizing effect was not observed in the reporter gene system suggests that the mechanisms involved are more complex than a simple stimulation of classical ER transactivation activity. Finally, the observation of this sensitizing effect suggests a hypothesis that exposure to low levels of weakly estrogenic pesticides in combination with an organophosphate pesticide might result in more breast cancer cell proliferation than would be expected by the organochlorine alone. The organophosphate compounds in this study are known to have antiandrogen activity. Considering that androgen agonists are known to inhibit estrogen regulated processes in some cells, it is reasonable that treatment with antiandrogens may relieve such suppression, resulting in a relative increase in organochlorine induced estrogen activity. The organochlorine compounds in the study are considered persistent contaminants with long elimination half lives. Thus, chronic exposure to low concentrations may have more estrogenic activity than would be expected if cells are sensitized or stimulated by periodic exposure to organophosphate pesticides. Contamination from older pesticides that are no longer used might be more significant if one is exposed to current use pesticides. See Y1 progress report for more information.

Year 2 activities related to Aim 1 included completing the matrix of pesticide combinations in the proliferation and MVLN reporter gene assays. Mixture combinations that produced the most dramatic sensitization effect in the breast cancer cells were selected for microarray analysis in Aim 2. These are: Parathion and opDDT, Fenitrothion and opDDT, and HPTE and opDDT.

Year Three Progress

In Year 3, we re-established the lab after Katrina and repeated experiments used to obtain the above results. This was an effort to validate that the new cell, new reagents and new lab conditions were able to produce the same results as obtained prior to Katrina. These experiments did correlate with previous findings.

Aim 2: Conduct cDNA microarrays to define a set of genes that are coordinately or differentially regulated by treatment with environmental hormones. Preparations from cells grown and exposed to mixtures of hormone active pesticides in the Wiese Lab will be evaluated for differential expression of genes in the Tulane Center for Gene Therapy.

(a). Identify target genes related to breast cancer cell proliferation from literature searches that will be used in gene array studies (Months 1−12).

(b) Prepare cells for gene array analysis after exposure to mixtures of pesticides. (Months

9−24).
(c) Run gene array analysis on cell preparations and analyze data (Months 12−36).

Year Three Progress
In Year 3 we obtained the equipment and prepaired the lab for the genomic phase of this project. To obtaine the equipment, Real Time PCR, a proposal was submitted to the Xavier College of Pharmacy and a BioRad iQ5 was purchased for use in the Wiese Lab for this project and subsequent use in Pharmacy teaching labs. The support of the college of provide this $36,000 instrument is significant. In additon, considerable time was spent testing RNA prep methods and developing 6 well plate cell seeding methods that would produce at least 10 ugms RNA. The Qiangen RNA prep method with the shredder and DNA removal was found to work well..

Aim 3: Confirm the expression pattern of genes identified by microarray through analysis of gene products (mRNA or protein).
(a) Select 6−10 genes that have been shown by differential display to have novel expression patterns as a result of pesticide mixture treatment (Months 14−36).
(b) Obtain probes for Northern blot analysis of selected genes (Months 14−36).
(c) Perform Northern blots to confirm expression observed in micro array studies (Months 24−48).
(d) Obtain antibodies for Western blot analysis of selected genes (Months 14−36).
(e) Perform Western blots to confirm expression observed in micro array studies (Months 24−48).

Year One Progress
No progress on this aim in year 3.

Deliverables/measurable outcomes:
Drs. Wang and Wiese will prepare or oversee the following:

1. Semiannual reports will be submitted to the PI.

Year Three Progress
These reports were submitted and have bee used to make this progress report.

2. Students involved in the research will present a poster at the annual research workshop (Months 12, 24, 36, 48).

Year Three Progress
In Y2, one Xavier undergraduate student was involved in research activities supporting the breast cancer project in the Wiese lab. Ashley White, a chemistry major and Xavier RISE scholar joined the Wiese lab in May 2004 and worked on this project through project Y1 and into Y2. Ashley has very good lab skills, was well trained in the lab and after graduation, she planned to continue working on this project for the next few yesrs while she was in pharmacy school at Xavier (started August 2005). However, Ashley did not return to Xavier after Huricaine Katrina. Another student will be selected to work on this project in the second half of 2006, after the lab is fully operational and after Xavier students are caught up with 2005-2006 school year courses running through the summer of 2006.

Dr. Wang will identify an undergraduate student to work on his project in the second half of 2006 or in early 2007. The method development required for his project in Y1 was not appropriate for student training. At the same time, the lab recovery process undertaking in first

half of 2006 is also difficult for student traiing because the students is not able to generate a product.

Both Dr. Wang and Wiese feel that the most effective student training only occurs when the full time staff members of the lab have sufficient expertise in the methodologies used in the lab. Thus, Y1 in both labs was dedicated to building expertise so that students in Y2 and beyond will have sold faculty and staff mentors from which to learn lab skills.

Drs. Wang and Wiese also have experience mentoring Xavier students in the MARC and RISE programs at Xavier. Once these programs are reestablished on campus in late 2006, both of Drs. Wang and Wiese will be bringing in at least one additional student into their labs.

3. One competitive grant application will be submitted by the end of the funding period.

Year Three Progress

Submission of a major equipment proposal to DoD for the acquisition of a tandem mass spectrometer.

In an effort to build up Xavier's capability to conduct independent proteomic research, Dr. Wang submitted a major equipment proposal to DoD's to the Army Research Office for consideration under ARO Broad Agency Announcement W911NF-05-R-0001 in 2004. The proposal was entitled "High Performance Liquid Chromatography-Tandem Mass Spectrometry for Enhancement of Teaching and Research at Xavier University" and asked for $196,392 for the purchase of an HPLC-MS/MS system. One of the major justifications for the proposal is the ongoing breast cancer research project for which the availability of such an instrument is essential. This proposal was not funded.

After returning to Xavier in January 2006 from the Katrina Evacuation, Drs. Wiese and Wang identified funds from 3 grants that could be rebudgeted to purchase the core components of the HPLC and Mass Spectrometry equipment requested in the above proposal. After, optaining approval from funding agencies, rebudgeting was done and the equipment was purchased in the first quarter of 2006. Thus, the core instrumentation equipment required to do proteomic analysis will be set up in Dr. Wangs lab in 2006.

Submission of a P20 planning grant to the NCI

A P20 planning grant was developed and submitted to the NCI in Y1. More details are provided in the Y1 progress report of Task 3 below.

4. Papers will be submitted to peer reviewed journals through the funding period.

Year Three Progress

No manuscripts were submitted in Y2.

Training deliverables:

1. The Tulane Cancer Center in conjunction with the Section of Hematology and Medical Oncology and The Cell Signaling group will be directly involved in providing breast cancer research training for Xavier Investigators.

Year Three Progress

The support provided from the TCC to each project is described within the progress reports of each project above. In addition, TCC support for the program as a whole is detailed in Task 3 below.

2. Toward the end of the project period, Drs. Wang and Wiese will be Co−PIs in writing an R01 grant in collaboration with Drs. Burow and Hill.

Year Three Progress
No R01 collaborative grants are in preparation at this time. We expect that during Y3, the planning for at least one collaborative grant will be started.

Task 2
Assist two Xavier junior faculty to become more competitive in breast cancer research

a. Identify two Junior Faculty with interest in breast cancer research (Month 1).

Year Three Progress
In the summer of 2004, Dr. Wiese and Dr. Klassen (coPI of the XU Prostate Training Grant) began identification of XU faculty that were interested in cancer research. This process resulted in one XU chemistry faculty, Dr. David Wolfgang, and one XU biology faculty, Dr. Mary Carmichael, submitting interest statements and CVs. Dr. Wiese then met with Dr. Hill to discuss potential mentors. Dr. Carmichael has been matched with Dr. Asim B. Abdel-Mageed from TCC on a Prostate Cancer project and is now developing a project proposal. Dr. Wolfgang was matched with Dr. Charles Miller at the Tulane Cancer Center in Spring 2005 and they were well along on developing a project when Katrina hit.

Efforts to get Xavier College of Pharmacy clincal faculty involved in developing a research project have not produced a viable team.

b. Establish participation of the selected Junior Faculty in Tulane Cancer Center seminars and the weekly signal transduction workshop focused on breast and prostate cancer (Month 2).

Year Three Progress
A regular group of XU faculty involved in the DOD Breast and Prostate cancer projects are attending the TCC and LSU CC seminars held most Thursdays at noon. Attendance has reduced after the Katrina evacuation since faculty are involved in additional responsibilities and lab recovery efforts. The LCRC signal transduction workshops have not yet started meeting. Dr. Wiese has discussed this situation with Dr. Hill who is in the process or reorganizing these workshops after the Katrina recovery. Xavier faculty involved in the DOD BC and PC programs have meet and decided to form a regular work in progress seminar meeting at Xavier starting in Fall 2006.

c. Determine Tulane Cancer Center mentors for the Junior Faculty and submit a two−page mini proposal for review of the PI and alternate PI (Month 6).

Year Three Progress
Dr. Wolfgang has been matched with Dr. Miller and they have developed the following project:

> **The Effect of Cellular Levels of the Hsp90 Co-chaperon p23 on the Stress Response of Mice Fibroblasts.**
>
> **Specific Aims:**

1) To determine if the amount of p23 in mouse fibroblasts affects the toxicity of anti-tumor compounds geldanamycin and herbimycin A.

2) To determine if the amount of p23 in mouse fibroblasts affects the toxicity of compounds (cadmium and arsenate) known to initiate the heat shock response.

Introduction:

Hsp90 is a chaperon protein that plays a role in the maintenance of steroid hormone receptors in their high affinity form. Hsp90 also interacts with kinases and polymerases. Proper Hsp90 function requires additional factors (co-chaperones) such as p23. Hsp90 has been linked to proteins involved in all six features found in almost all cancers; 1) self sufficient growth signaling, 2) insensitive to signals that halt the cell cycle, 3) evade apoptosis, 4) angiogenesis, 5) metastasis, and 6) unlimited potential for cell division. As such the understanding of Hsp90 and its co-chaperones is vital to understanding and treating cancer. The protein p23 has been shown to maintain Hsp90 in its active, ATP bound, form. p23 is also part of the Hsp90 complex that is involved in chaperoning estrogen receptor alpha. The fact that p23 is up-regulated in cancer cells suggests that it may play a role in tumor growth. It has also recently been shown that overexpression of p23 in MCF-7 cells enhances adhesion and invasion. Dr. Charles Miller has mice that are heterozygous for the p23 gene, one copy has been knocked-out. We must obtain cells from embryos since mice that are p23 homozygous null are not viable beyond birth.

Methods:

Mice heterozygous for the p23 gene are mated and females are sacrificed about a day before birth. Skin cells are plated in T25 flasks with DMEM media supplemented with 10% Fetal Bovune Serum, L-glutamine, sodium pyruvate, and antibiotics. When the plates are confluent the cells are treated with trypsin to remove them from the flask, counted, diluted to 12,000 cells per mL, and seeded in 96-well plates at a concentration of 1200 cells per well. These cells are grown overnight at 37ºC. Doses of the test compound are diluted in DMEM media supplemented as described. Geldanamycin and Herbimycin are tested in the range of 10 μM to 1 nM final concentration. Cadmium and arsenate were tested in the range of 0.3 to 10 μM final concentration. The old media is suctioned out of the wells and replaced with 100 μL of media with the appropriate dose. Three sets of controls and prepared; wells with cells and "dosed" with media only function as a positive control, wells without any cells function as a negative control, and cells "dosed" with media and DMSO control for the fact that Geldanamycin and Herbimycin A are diluted from a stock solution dissolved in DMSO. The dosed cells are returned to 37ºC and incubated for 24 hours, after which the dose is suctioned off and replaced with media. The plates are then returned to 37ºC for three days. At the end of the three days 10 μL of 0.1% (w/v) alamar blue (resazurin) diluted in phosphate buffered saline is added. The plates are returned to 37ºC to allow the surviving cells to reduce the alamar blue into resorufin. The resorufin fluoresces at 590 nm and this fluorescence is measured usually at 6-8 hours after addition of alamar blue. The fluorescence from the positive control is set at 100% and the fluorescence from the negative control is set at 0 %. The data is fit to a sigmoidal dose response curve and a concentration that yields 50% fluorescence is expressed as the EC50 value.

Results:

Initially tests were run using an immortalized cell line of p23 null mouse fibroblasts. This was done in anticipation of transfecting the p23 gene into these cells and doing the comparison between these cell lines. However, the stable transfection of the p23 gene was unsuccessful. These experiments served to determine the appropriate range of concentrations to use. They also gave an indication of the reproducibility of the experiments.

Table 1. EC50 values for immortalized p23 null cells.

Compound	EC50 values	Mean +/- SD
Geldanamycin	18, 19, 15, 43 nM	24 +/- 14
Herbimycin	900, 630, 1038, 1427 nM	1000 +/- 330
CdCl2	2.9, 4.2, 4.4, 3.6 μM	3.8 +/- 0.68
Sodium Arsenate	140, 110, 110, 97 μM	110 +/- 18

Experiments were conducted using immortalized cell lines of WT and p23 null cells. There were no significant differences, for example using $CdCl_2$ the measured EC50 values were 2.75 +/- 0.79 μM (n = 4) for p23 null verses 2.35 +/- 0.81 μM (n = 7) for WT and heterozygous.

At this point it was decided to test primary fibroblasts. A few matings failed to yield any p23 null embryos. One mating produced four WT, three Heterzygotes, and three Null embryos.
The data from these mice is summarized in the following table:

Table 2. EC50 values.

Test compound	WT EC50 n=4	HZ EC50 n=3	Null EC50 n=3
Geldanamycin	12.4 +/- 5.32 nM	9.0 +/- 3.61 nM	5.9 +/- 2.74 nM
Herbimycin A	972 +/- 646 nM	629 +/- 440 nM	254 +/- 165 nM
$CdCl_2$	5.65 +/- 1.0 μM	6.01 +/- 1.34 μM	3.93 +/- 0.40 μM
Sodium Arsenate	232 +/- 70.5 μM	246 +/- 91.5 μM	112 +/- 23.5 μM

The differences for sodium arsenate and CdCl2 are statistically different to a p value of 0.05. The differences for geldanamycin and herbimycin A are not significant due to the large standard deviations: however, these compounds show the expected pattern of less p23 and a lower EC50 value (figures 1 and 2)

Figure 1. EC50 values for Geldanamycin.

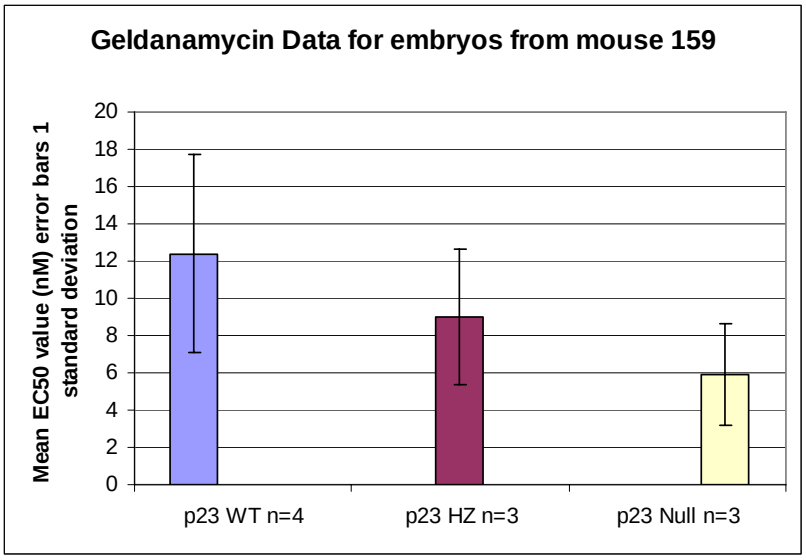
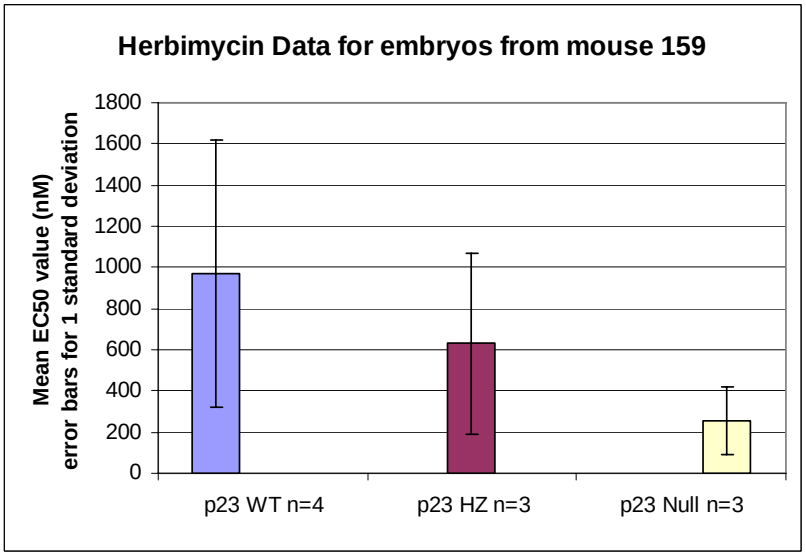


Figure 2. EC50 values for Herbimycin A.



Future Plans:

Three more breeding pairs have been set up in order to repeat the experiment in Table 2. There is also concern that having the cells growing in culture for any length of time prior to exposure to the test compounds selects for cells that are able to survive stress conditions without p23. We are concerned that we are selecting against the cells that are most susceptible to the compounds. In order to limit this we will also take cells directly from the embryo place them in the 96-well plate and dose them directly before there has been any time for selection. Another possible approach to limiting stress would be to grow the cells in a reduced oxygen environment.
Another avenue of investigation could be to investigate how these p23 null cells survive at all. A proteomics approach could be used. By running 2-dimensional gels it would be possible to compare the protein expression in WT cells and p23 null cells. Perhaps there is an up-regulation of some other protein in the p23 null cells that allows them to survive.

The interaction between myself and Dr. Miller was primarily by e-mail (on average about once a week) and occasionally by phone. We presented a combined presentation to the other Xavier Tulane researches in the monthly DOD Research Meeting. Without the demands of teaching the amount of interaction will be greater during the summer.

d. Junior Faculty collect preliminary data (Months 7−36).

Year Three Progress
See Task 2 c for current status of the Wolfgang-Miller project.

e. Junior Faculty develop grant proposal (Months 36−48).

Year Three Progress
No Progress in this area in Y1.

Task 3
Establish infrastructure that will create an environment that fosters breast cancer research, in which Xavier faculty will receive substantive training and become more competitive for DoD funding

Background and Year Three Progress
When Xavier was awarded the DOD Breast Cancer grant in April 2004, Dr. Rosenzweig, the project PI, announced that she would leave Xavier in May 2004. A plan was formulated where Dr. Wiese, PI of one of the research projects in the Breast Cancer training program would take over program PI responsibilities along with his research project. He would be provided release time for both tasks and be assisted by a part time administrative assistant that would be hired. Dr Wiese served 5 years as a joint faculty between Tulane and Xavier before moving full time to Xavier in 2003. While at Tulane, he became a member of the Tulane Cancer Center and developed a good working relationship with Dr. Steven Hill, Tulane coPI of this project. Dr. Wiese also had also developed a good working relationship with Dr. Klassen, Xavier Chemistry Department, coPI of the XU-YU DOD Prostate Cancer training program, when Dr. Klassen utilized the cell culture facilities in the Wiese lab in 2003-2004.

Unfortunately, Dr. Klassen elected not to return to Xavier after Katrina. Dr. Wiese, the PI of the Xavier DOD BC program has been asked by the Xavier administration to replace Dr. Klassen as PI of the Xavier DOD Prostate program effective Feb 2006.

The Xavier DOD BC and PC programs continue to operate in parallel with meetings, seminars and discussion sessions involving both groups. In Y1, we established an email list serve for all Xavier and Tulane faculty involved in both XU DOD cancer training projects and this mechanism has been very helpful for rapid communication of cancer center events, project meetings and organizing car pools to LCRC seminars.

It should be noted that the Tulane Cancer Center is part of the Louisiana Cancer Research Consortium (LCRC) that includes the LSU Cancer Center. The LCRC was devised in 2002, involves significant funding from the state of Louisiana and will eventually be housed in a new building between the Tulane and LSU medical centers in New Orleans. The LCRC is co-directed by Dr. Roy Weiner (Director of Tulane Cancer Center) and Dr. Oliver Sartor (Director of the LSU Cancer Center). Drs. Klassen and Wiese were invited to the first annual LCRC

retreat in January 05. The planning process and meetings that took place at this retreat clearly stated that all Xavier faculty interested in or doing cancer research were welcome to participate in the LCRC through adjunct appointments in Tulane or LSU departments. In addition, Dr. Roy Weiner has keep in close contact with Dr. Wiese regarding the Xavier DOD Breast Cancer training program and has made it clear that he is personally committed to helping Xavier faculty develop cancer research projects and programs. He has opened up all the resources of the Tulane Cancer Center core facilities to Xavier researchers and has invited Xavier faculty to be involved in the Tulane Cancer Centers cancer research symposia held each fall. This Mauvernay Research Excellence Award program includes seminars and posters related to cancer research and concludes with a dinner where TCC faculty meet the invited speakers. Several of the XU faculty involved in the DOD cancer training programs attended the Mauvernay Research Excellence Award program in fall 2004 and Drs. Hill and Weiner made a special effort to introduce the XU faculty to TCC faculty and to the invited speakers. Dr. Weiner also has included clinical faculty from the Xavier College of Pharmacy in ongoing initiatives at the Tulane Cancer Center.

One result of this close relationship between Drs. Weiner and Hill of the TCC and Xavier University is the submission of a P20 planning grant to the NCI in February of 2005 (see Abstract in Y1 Progress Report Appendix). This grant is specifically designed to plan long term collaborations between cancer centers and minority serving institutions. Through a series of meetings starting in October 2004, a P20 grant was developed between the Tulane Cancer Center and Xavier University with Dr. Weiner as the Tulane PI and Dr. Kathleen Kennedy, Associate Dean, Xavier College of Pharmacy as the Xavier PI. At the same time, the PI and co-PI of the Xavier DOD Breast Cancer Training Program, Drs. Wiese and Hill became the P20 grant program managers for each respective institution. Drs Wiese and Hill also took responsibility for the majority of the organization, planning and preparation of this panning grant over a 5 month period leading up to submission in February 2005. This NCI P20 program grant was awarded in August 2005 with a start date of October 1, 2005 (during the Katrina evacuation). The good working relationship of Dr. Wiese and Hill, developed largely from the DOD Breast Cancer Training Program and other prior activities, was critical to working out the complex details of this P20 proposal that involved two very different universities. We feel that the DOD cancer training programs between Tulane and Xavier provided the critical mass required to put together this P20 grant and that the combination of these programs will contribute significantly to the development of self sustaining cancer research programs at Xavier in the future.

The review of the Xavier DOD Breast Cancer Training program requested that an administrative assistant be hired to assist the PI in grant management tasks as well as in planning meetings and coordinating communication between all those involved at XU and TU. In August 2004, Mr. Sergio Alcantera was hired as a part time program manager for this project. See Y1 progress report for more details. Mr. Alcantera moved his family to California after Katrina leaving this position open.

With the award of the NCI P20 training grant in 2005, Xavier now had two program grants that had openings a program assistant. With the help of Dr. Roy Weiner at the Tulane Cancer Center and a search process at Xavier, a sutable candidate was identified in early 2006. Ms. Stephanie Colbert was hired by Xavier in February 2006 to support both the DOD BC program and the NCI P20 grant working under the supervision of Dr. Wiese, PI of the DOD BC program and manager of the NCI P20 program. For Ms. Colbert's CV, see Appendix 4.

a. Grant membership in the Tulane Cancer Center to Xavier researchers. Drs. Wang and

Wiese will be granted a status of contributing members and the junior faculty will be granted a status of associate members. Please see attached TCC publication for the definitions (Month 1).

Year Three Progress
At this time, only Dr. Wiese has formal membership in the Tulane Cancer Center because he is an adjunct faculty in the Biochemistry Department of the Tulane school of Medicine. Dr. Hill at Tulane is now working on a mechanism to bring adjunct appointments to Xavier faculty involved in collaborative projects with the Tulane Cancer Center.

b. Include Xavier researchers in Tulane Breast Cancer focus group and Journal Club (Months 2).

Year Three Progress
The Tulane Cancer Center Focus Groups are now combined with the LSU Cancer center under the LCRC. At the LCRC retreat in January 2004, the following focal groups were established: Molecular Genetics, Molecular Signaling, Immunology, Epidemiology and Clinical Research. To date, while membership in these groups as been established, regular meetings of these groups has not occurred. Xavier cancer research faculty have decided to hold their own research in progress meetings that include Tulane collaborators starting in Fall 2006.

c. Grant access to core research facilities at Tulane Cancer Center (Month 1).

Year Three Progress
Access to TCC and LCRC core facilities has been granted to Xavier faculty. These cores include: Genomics, Proteomics, Biostatistics/Bioinformatics, Immunology, and Tissue Acquisition. To date, no Xavier faculty have required the use of these facilities.

d. Include a student in each research project (Month 2 for Drs. Wang and Wiese and Month 8 for the junior faculty).

Year Three Progress
See Task 1 above.

e. Establish a monthly brown−bag lunch meeting to bring up research related issues, review proposals and papers, or brainstorm on new directions to improve the cancer program (Month 1).

Year Three Progress
Due to the busy schedules of both TCC and XU faculty involved, monthly meetings have proven to be difficult to organize. We did hold an organizational meeting in February 2006 after the evacuation. In this meeting we determined that all program participants were still interested in continuing the program and that the appropriate Tulane mentors were also available and interested in continuing with the program. We also determined that with the lack of Tulane Cancer Center research group meetings (have yet to meet), we would establish a research in progress lunch presentation-discussion meeting series in Fall 2006 after the post-Katrina teaching loads returned to normal (see Task 3 b above).

e. Hold an annual workshop, open to all in the Xavier and Tulane communities and Xavier student body, for all BC participants to present results of the preceding year. Faculty, students, and staff will attend and at least one person from each group will present a

talk; students will present posters (Months 12, 24, 36, 48).

A. First workshop titled "Molecular Signaling in Breast Cancer" (Month 12).
B. Second workshop titled "Breast Cancer and the African American Community" (Month 24).
C. Third workshop titled "Funding Opportunities in Breast Cancer Research" (Month 36).
D. Forth workshop titled "Drug Design and Delivery in Breast Cancer" (Month 48).

Year Three Progress
Plans to hold a joint Breast and Prostate symposia are currently on hold. We expect to revisit planning for this activity in the Fall of 2006.

f. Subscribe to breast cancer related journals (Month 1).

Year Three Progress
In Y1, we purchased a subscription to the online journal Breast Cancer Research. In Y2, we determined that we could get access to the journal Proteomics through the Xavier Library and that a subscription was not needed. Access to Tulane library resources is still limited for XU faculty. Only faculty with adjunct appointments have off campus online access. Dr. Wiese has access (from prior adjunct appointment) and is serving as the access point for journal articles needed from Tulane. In Y3 we must establish XU faculty as adjuncts at Tulane to resolve this problem.

Key Research Accomplishments

- We have shown that over expression of MEK5 increases breast cancer tumor volume independent of estrogen.
- Proteomics capabilities have been set up at Xavier and are now being used to characterize the MEK5 signaling pathway.
- We have shown that the combination of organophosphate and organochlorine pesticides can interact to enhance the estrogen activity of the organochlorine.
- A new project is being developed to study the role of P23 in HSP90 control of cell signaling.
- All members of this research team have returned after the Katrina evacuation and are in the process of re-establishing their research programs.

Reportable Outcomes

1. MCF-7 MEK5 cells that stably express MEK5.
2. Proposal entitled “High Performance Liquid Chromatography-Tandem Mass Spectrometry for Enhancement of Teaching and Research at Xavier University”, $196,392, DoD ARO Broad Agency Announcement W911NF-05-R-0001. Not Funded.
3. Proposal entitled “Planning Grant Minority Institution/Cancer Center Collaboration”, $703,574, NIH NCI RFA-CA-05-020. Funded 2005-2009.
4. A $25,000 recovery grant was awarded to this research team by the Tulane Cancer Center and the LCRC to rebuild programs after Katrina.

Conclusions
We have established two collaborative breast cancer research projects and are in the process of building one more new project. We have built a framework of activities for XU faculty to utilize for interaction with the TCC/LCRC to develop cancer research initiatives involving Xavier undergraduate and pharmacy students. Most importantly, we have maintained our program team after the Katrina evacuation and are in the process of re-establishing our research programs.

Year Three Synergy and Opportunities
In Y3, we plan to build on established interactions with the XU-TU DOD Prostate Cancer program. The main goal of Y3 will be to re-establish our research programs after the Katrina evacuation. We will request a funded, one year extension from the DOD in Y3. We also plan on building our small cancer research network at Xavier by holding regular cancer research discussion meetings and developing the aims of the NCI P20 grant funded in 2005. Our long term goal is to establish a core of faculty at Xavier that are active in cancer research and education.

Year Three Challenges
Year 2 has more challenge than could be anticipated. We must re-establish our research labs, validate experiments and pre-Katrina research findings and then move forward on the next stages of our research. The immediate challenge is that all Xavier faculty have higher teaching loads (faculty was downsized) and that the summer of 2006 will be spent teaching to catch students up after the lost time during the Katrina evacuation. Taken together, these tasks will impact the DOD program in at leas the following ways: 1. Time must be spent to get labs up a going again, 2. Less time can be spent on research or rebuilding labs because of increased teaching, 3. All faculty and staff in the project are also dealing with displaced families, the loss of homes and the search for new living arraignments in New Orleans.

References
NA

Appendices

Appendix 1

Developing Breast Cancer Program at Xavier:
Genomic and Proteomic Analysis of Signaling Pathways Involved in Xenohormone and MEK5 Regulation of Breast Cancer

HBCU/MI Partnership Training Award:
Xavier University of Louisiana and the Tulane Cancer Center
Proposal Number: BC030300
Fund Period: 2003 (April 2004-April 2008)
Principal Investigator: Thomas Wiese (Replacing Nitza Rosenzweig in 2004)
Task 1: Complete 2 Breast Cancer Pilot Research Projects
Thomas Wiese (Xavier Pharmacy), Steven Hill (Tulane Cancer Center)
Guangdi Wang (Xavier Chemistry), Matthew Burow (Tulane Cancer Center)
Task 2: Involve 2 Junior Faculty in Breast Cancer Research
David Wolfgang (Xavier Chemistry), Charles Miller (Tulane Cancer Center)
Mack Crayton (Xavier Biology)
Task 3: Establish Environment at Xavier that Fosters Breast Cancer Research
Seminars, Workgroups, Symposia, Student Research Training
Synergy: Xavier-Tulane DOD Prostate HBCU Collaborative Partnership Award PC030945, Feb 2004-Jan 2007, Training HBCU Faculty and Students in Prostate Cancer (PC) Research: Signal Transduction and Receptor-Inhibitor Interactions in the Progress of PC

Mission of Xavier University of Louisiana

African American and Catholic

Work toward a more just and humane society:

- Education
- Service
- Research

Examples of Success

- pre-Med Students
- Pharmacists
- pre-Biomedical Graduate Programs

The Katrina Effect

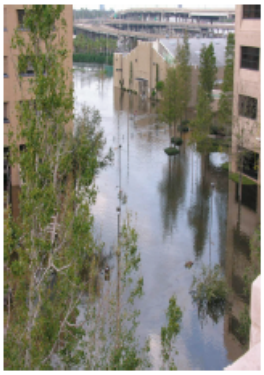
1. **Damage to Xavier and Tulane**
   a) Facilities
   b) Equipment
   c) Loss of People
   d) Loss of Homes: Faculty and Staff
2. **Some Research Activities During Evacuation**
3. **Xavier and Tulane are Back (close to normal)**

The Katrina Effect

1. **Facilities: Xavier University**
   a) 1-6 feet of nasty salt water for 3 weeks
   b) All first floors of buildings gutted and remodeled
   c) All new electrical, phone and internet campus wide
   d) All new mechanical rooms in all buildings
   e) New Physical plant
2. **Campus Operational Jan 9 2006**
3. **Fall Semester Resumed Jan 19**
   a) More than 75% students returned (more than 90% in Pharmacy)
4. **$40 million spent to date (expect to spend $45 million)**
5. **FEMA and Insurance to pay 90%**
6. **$15 million from donations to date**

The Katrina Effect

1. **Facilities: Tulane Medical Center**
   a) 1-3 feet of nasty salt water for 2 weeks
   b) All first floors of buildings gutted and remodeled
   c) Some new electrical, phone, internet campus wide
   d) Some new mechanical rooms and other rebuilding
2. **Campus Operational December 2005**



Science Quad During Katrina

Science Quad March 2006

NCF 105
October 2005

NCF 105
March 2006

The Katrina Effect: Post Katrina Reality

1. **Teaching**
   a) Tulane faculty travel to Houston to teach (till August 2006)
   b) Xavier faculty teach heavier load all summer 2006
2. **Research**
   a) Lost time in reestablishing labs
   b) Lost supplies and equipment
   c) Some lost release time for 2006
3. **Feeling on Both Campuses**
   a) Positive, but stressed…
   b) Tenure and Promotion Reestablished
   c) Salary Increases for 2006-07 contracts

The Katrina Effect on DoD Cancer Projects

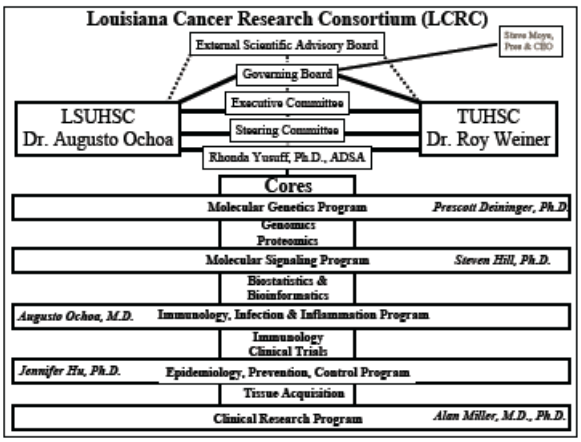
1. **Asking for supplemental funding**
   a) Supplies
   b) Equipment
   c) Personnel
2. **Asking for a 1 year extension**

Evaluation of Collaborative Partnership

1. **How did the partnership originate?**
   a) DOD Breast Cancer HBCU/MI-Focused Training Award 1999: Nitza Rosenzweig (original Xavier PI) and Kim O'Connor (Tulane Cancer Center)
   b) Tulane-Xavier Joint Faculty Appointment Program: Thomas Wiese 50% Xavier and 50% Tulane 1998-2003
   c) Interest in Cancer Research at Xavier
   d) Tulane Cancer Center Interest in Xavier Collaboration
   e) History of other research collaborations

Cancer in Louisiana

1. **Nation's Highest Death Rate from Cancer**
2. **4th Lowest Compliance with Mammography**
3. **Most Cancer Care is Fragmented**
4. **No NCI-Designated Cancer Center in La.**
   a) None in Mississippi or Arkansas
   b) Of 61 centers in US - Closest is 250 mi. Away



Tulane Cancer Center-LCRC Goals

1. **Build Research Programs**
   a) Recruit funded faculty to Programs
   b) Expand funded research of resident faculty
2. **Enhance Education**
   a) Develop new courses by new faculty
   b) Upgrade graduate students and trainees
3. **Expand Clinical Access**
   a) Enlarge clinical research faculty
   b) Offer innovative translational research
4. **Merit NCI Center Designation (2010?)**

Evaluation of Collaborative Partnership

2. **Is there evidence of a productive relationship between the collaborators?**
   a) **Research progress**
   b) **Abstracts: Era of Hope, Keystone**
   c) **Manuscripts in Prep**
   d) **Weekly seminar series**
   e) **Bimonthly LCRC focus group meetings**
   f) **Biweekly Breast Cancer Research Workshop**
   g) **Xavier Included in LCRC Planning Retreat**
   h) **Xavier President on LCRC Board**

Abstracts After Year One

1. Virgilio A. Salvo, Matthew E. Burow, et. al., **MEK5-Erk5 signaling promotes estrogen-independent tumorigenesis of breast carcinoma cells**. Keystone Symposia, Hormonal Regulation of Tumorigenesis (B6), February 20-25, 2005, Monterey, CA. [Selected for Oral Presentation- Nuclear Receptors an their Co-regulators, Feb 23rd]

2. Wiese TE, Li H, Nguyen HM, Hill SR (2005) **INTERACTIONS OF ESTROGEN AND PROGESTIN ACTIVE ENVIRONMENTAL CHEMICALS ON BREAST CANCER CELL PROLIFERATION, SURVIVAL AND GENE EXPRESSION**. *Era of Hope 2005, June 8-11, 2005, Philadelphia, PA*

Evaluation of Collaborative Partnership

3. **Have collaborators secured additional funding based on their scientific partnership?**

- **After Year 1.5**
  a) **Individual project grants planned (delayed by Katrina)**
  b) **LCRC provided $25,000 Post Katrina Support to Xavier Cancer Researchers**
  c) **Program grant submitted 2005 and funded: NCI P20**

Xavier – Tulane NCI Planning Grant for Minority Institution/Cancer Center Collaboration (P20)

- PI's: Dr. Kathleen Kennedy, Xavier University
  Dr. Roy Weiner, Tulane University
- coPIs/ Managers: Dr. Thomas Wiese, Xavier University
  Dr. Steven Hill, Tulane University
- Funding Period: October 2005 thru September 2009

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1. Collaborative Research Pilot Projects

a) XU and TU faculty working together (collaboration or mentored relationship)
b) $100,000 for 3 years ($50,000 from P20 and $50,000 from LCRC)
c) Focus on cancer and minority health/health disparities

2. Undergraduate Research

a) 2 XU undergraduates working in labs of XU or TCC mentors
b) Supplies and travel funds

Xavier – Tulane NCI Planning Grant for Minority Institution/Cancer Center Collaboration (P20)

3. Cancer Biology Course including Cancer Health Disparities Aspects

a) Introduction course, first course in Cancer Biology
b) XU and TU faculty instructors (using synchronous distance learning)
c) XU and TU students (undergrad, graduate, professional: PharmD and MD)

4. Cultural Competence and Minority Health Disparities Education

a) Xavier Institute for Health Disparities and Minority Health Education instructors
b) Students from XU and TU communities (Faculty, Staff, Clinical Staff, Students, etc.)
c) Issue-focused seminars, problem-based group activities, Web resources, etc.

Evaluation of Collaborative Partnership

4. Have the collaborators established additional scientific partnerships with other institutions?

a) **Children's Hospital**
b) **New collaborations within Xavier (Wiese-Wang)**
c) **LCRC: Tulane and LSU Med Center Linkages: Core Facilities**

Evaluation of Collaborative Partnership

5. Has the HBCU/MI PI initiated additional scientific collaborations within the institution?

a) **Wiese Lab and Wang lab**
b) **Linkages with Xavier DOD Prostate Grant**
c) **Core of Xavier Faculty Interested in Cancer Research**

Evaluation of Collaborative Partnership

6. Is your research competitive with the current field of study?

- **Building capabilities:**
  a) Cancer Research Expertise
  b) Linkage to LCRC and TCC facilities
  c) Instrumentation at Xavier

Evaluation of Funding Activities

1. Has PI/laboratory secured independent funding since BCRP award?

- **Not yet, but working toward:**
  a) **Xavier-Xavier Collaborative Proposals**
  b) **Xavier-Tulane Collaborative Proposals**

Evaluation of Funding Activities

2. **Has PI secured funds with collaborating partner or other collaborators?**

- **Yes: NCI P20**
  a) **Permanent Xavier Linkage to Tulane Cancer Center**
  b) **Xavier as working partner in LCRC**
  c) **Xavier and Tulane linked by Cancer Research and Education**

Evaluation of Infrastructure Development

1. **What is the state of research facility?**

- **Xavier labs well equipped and functional**
  a) **Before and After Katrina**
  b) **Some repairs and replacements underway**

Evaluation of Infrastructure Development

2. **Were PI/ Partnership responsible for additional infrastructure?**

- **Yes!**
  a) **Provided critical mass…**
  b) **Biweekly Breast Cancer Research Workshop**
  c) **Inclusion of Xavier faculty in LCRC Seminars**
  d) **Xavier faculty access to LCRC cores**
  e) **Proteimics Mass Spec Facility**
  f) **LCRC Provided $25K post-Katrina aid to Xavier Breast Cancer Researchers**

Evaluation of Mentorships

1. **How many H.S. students, undergrads, predocs and postdocs have been developed because of funding?**

- **Xavier Undergraduates**
  a) Two in Wiese lab: Ashley White, Hasina Ashe
  b) One to three Planned for Wang Lab (once methods developed)

- **Xavier Pharmacy Students**
  a) Two in Wiese Lab: Julie Nguyen, Mia Louis

Evaluation of Mentorships

2. **Has the partnership resulted in the production of new breast cancer scientist?**

- **Guangdi Wang (Xavier Chemistry)**
  a) Analytical Chemistry Focused on Cancer
- **David Wolfgang (Xavier Chemistry)**
  a) Developing project with Charles Miller (TCC)
- **Mack Crayton (Xavier Biology)**
  a) Evaluating mentors and projects

Evaluation of Mentorships

3. **How is the collaborating institution contributing to the HBCU/MI institution's success?**

- **Mechanisms established for Xavier faculty using Tulane Cancer Center and LCRC facilities.**

- **Xavier involvement in TCC and LCRC seminars, cancer workshops, program planning**

Evaluation of Training

1. **What approaches have been undertaken to train the supported researchers at HBCU/MI institution?**

- **Mechanisms established for Xavier faculty using Tulane Cancer Center and LCRC facilities.**
- **Xavier involvement in TCC and LCRC seminars, cancer workshops, program planning**

Evaluation of Training

2. **Have new training programs occurred as a result of funding?**

- **Xavier-Tulane NCI P20:**
  a) Cancer Research: Pilot Projects
  b) Cancer Education: Cancer Course, Cultural Competence Courses

Pilot Project #1

Chemoresistance in Breast Carcinoma Cells: MEK5-BMK/Erk5 Expression and Proteomic Analyses

PI: Guangdi Wang, Ph.D.
Xavier University of Louisiana

Co-PI: Matthew Burow, Ph.D.
Tulane Cancer Center

OBJECTIVES

1. **Implication of the role of MEK5 in survival signaling and suppression of apoptosis in breast carcinoma cells.**

2. **Characterization of proteomic differences between resistant and sensitive breast carcinoma cells and identification of anti-apoptotic proteins within MEK5-expressing cell lines**

The Collaborators

Guangdi Wang's Lab (Xavier University)
Proteomic Analysis Development
2D Gels
HPLC-MS/MS (future)

Matthew Burow's Lab (Tulane Cancer Center)
Breast Carcinoma Cell Model
Molecular and Functional Characterization

Yang Cai's Lab (Children's Hospital Research Center)
Gel Spot Cutter
Auto-digestion Station
2D-HPLC-MS/MS Instruments

Specific Aims

Aim 1- to demonstrate the requirement for and role of the MEK5 pathway in survival signaling and suppression of apoptosis in breast carcinoma cells.

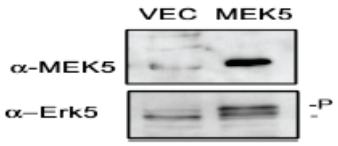
Aim 2- to characterize proteomic differences between MCF-7N and MCF-7M breast carcinoma cells, to identify anti-apoptotic proteins within MEK5-expressing cell lines and to compare MEK5 expression and proteomic variants in drug resistant breast cancer cells derived from MCF-7 and ZR-75-30, respectively.

Aim 3- Validation of proteomic target identification in resistant breast carcinoma cells.

Progress Highlights: Cancer Biology

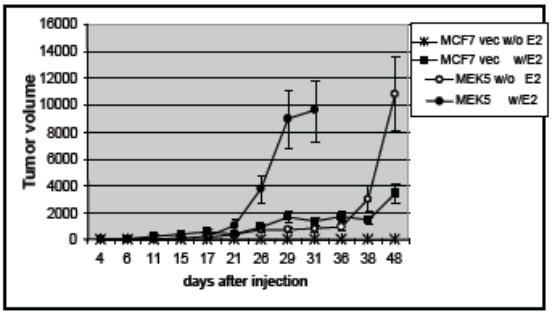
1. Generation of stable MCF7N-CA-MEK5 cells
2. MCF-7 tumor formation in *Scid*/Beige mice
3. Stable expression of Erk5-RNAi in MCF7-MEK5 cells

Generation of stable MCF7N-CA-MEK5 cells

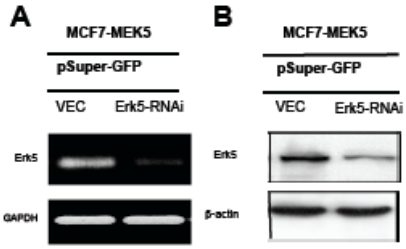


Generation of stable MCF7N-CA-MEK5 cells. Stable clones of MCF7N cells expressing either constitutively active MEK5 (MCF7N-MEK5) or vector (MCF7N-VEC) were examined by western blot analysis for expression of MEK5 (upper panel) or Erk5 (bottom panel).

MCF-7 tumor formation in *Scid*/Beige mice

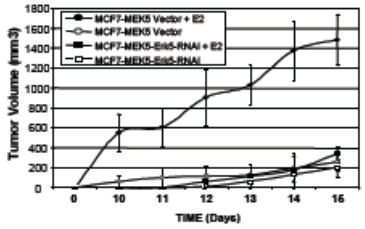


Stable expression of Erk5-RNAi in MCF7-MEK5 cells



MCF7N-MEK5 cells were transfected with pCMV-hygro along with either empty pSUPER-GFP (pSUPER-VEC) or pSUPER-GFP containing Erk5 siRNA sequences (pSUPER-Erk5-RNAi) and stable clones were selected in hygromycin. Decreased expression of Erk5 was confirmed with PCR (A), and western blot analysis (B).

Erk5-RNA interference partially suppresses MCF7-MEK5 tumor growth



MCF7N-MEK5-(VEC) or MCF7N-MEK5-(Erk5-RNAi) cells (5 X10^6) were injected (s.c.) into the flanks of SCID-Beige mice either in the presence (+E2) or absence of slow release estradiol pellets (0.72 mg, 60 day release) (n=5 /group). Tumor growth was monitored daily after palpable tumor formation and was represented as tumor volume (mm^3) ± S.E.M. (n=5).

Impact of Hurricane Katrina

Loss due to water damage and power outage:

- Cell lines saved
- $20,000 – Reagents and supplies

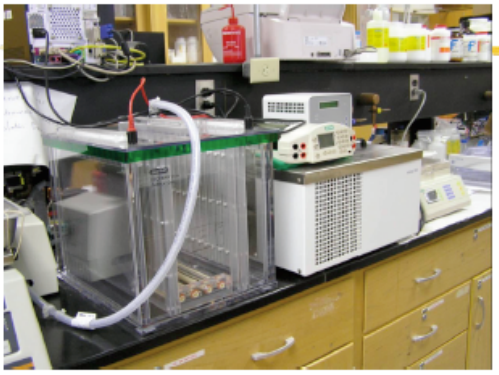
Loss in time and research productivity

- 6 months – 12 months
- Burow lab at Baylor and Tulane (moving back to Tulane)

Progress Highlights: Proteomics

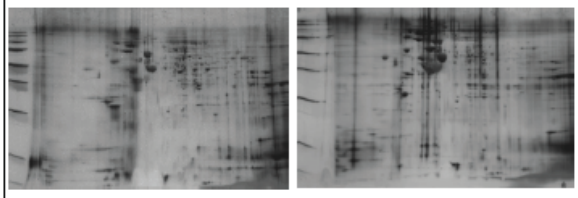
1. **Developed and optimized methods for**
   - Protein extraction from whole cells
   - Gel spot isolation/cutting
   - Protein digestion
   - 2-D HPLC and MS/MS separation and identification of peptides
2. **Obtained preliminary gel images of MEK5 expressing MCF-7N cells and normal MCF-7N cells**
3. **Started growing MCF-7N-MEK5 and MCF-7N-VEC cells in Wiese's lab at Xavier University**

2D-Gel System – Wang's Lab at Xavier



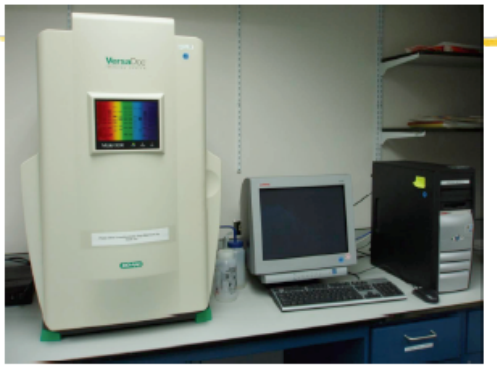
Gel Imaging System – Wang's Lab at Xavier

Progress Highlights: Proteomics



2D gel electropherogram of protein extracts from (A) MCF-7N-MEK5 cells and (B) MCF-7N-VEC cells using pH 3~10 IPG strips and 11 cm 8~16% linear gradient SDS PAGE gel, and silver stain

Gel Imaging System – Children's Hospital



Gel Spot Cutter – Children's Hospital

Protein Digest Station – Children's Hospital

2D-HPLC-LTQ MS/MS – Children's Hospital

Impact of Hurricane Katrina

Loss due to water damage and power outage:

- $18,000 – Reagents and supplies
- $ 4,500 – Small equipment and a computer

Loss in time and research productivity

- 6 months – 12 months
- Instruments at Children's Hospital are still not fully functional

Ongoing and Planned Activities

1. Purchase of a 2-D HPLC-MS/MS system

- Rebudget of DoD funds for partial contribution to the purchase
- Enhance research productivity
- Provide protein MS capability at Xavier and TCC

2. Set up cell culture experiments at Xavier (Wiese Lab)

- Expedite sample procurement
- Develop expertise and collaboration among Xavier cancer researchers

Pilot Project #2

Interactions of estrogen and progestin active environmental chemicals on breast cancer cell proliferation, survival and gene expression

PI: Thomas E. Wiese, Ph.D.
Xavier University of Louisiana

Co-PI: Steven R. Hill, Ph.D.
Tulane Cancer Center

OBJECTIVES

Characterize the interactive effects of binary mixtures of DDT and methoxychlor isomers and metabolites with estrogen and/or antiprogestin activity on:

•Breast Cancer Cell Proliferation

•Gene Induction

The Collaborators

Thomas Wiese Lab (Xavier University)
Cell Models: Proliferation and Reporter Gene
Cell and Molecular Analysis of Genes and Gene Products

Steven Hill Lab (Tulane Cancer Center)
Experimental Design and Analysis
Microarray Design and Analysis
Strategy for Molecular Characterizations

Tulane Cancer Center
Microarray Facility (Gene Therapy)

Specific Aims

Aim 1- Examine the effects of binary mixtures of estrogen and progestin active environmental compounds on cell proliferation and survival.

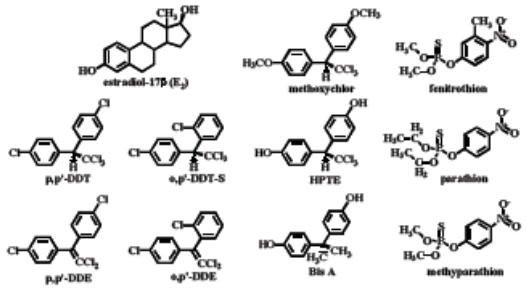
Aim 2- Conduct cDNA microarray analysis to define a set of genes that are coordinately or differentially regulated by treatment with environmental hormones.

Aim 3- Confirm the expression pattern of genes identified by microarray through analysis of gene products (mRNA or protein).

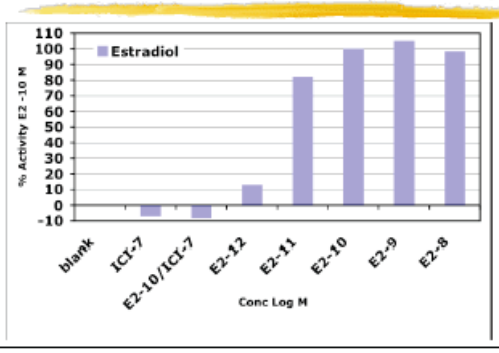
Hypothesis and Relevance

1. **The estrogen regulated proliferation of breast cancer cells treated with pesticide mixtures will be different than observed when cells are treated by individual pesticides alone.**
2. **Human exposure to hormone active pesticides includes multiple chemicals of multiple types.**
3. **This study explores the interaction effects of DDT like organochlorine pesticide isomers and metabolites with organophosphate pesticides on breast cancer cell proliferation.**
4. **The isomers and metabolites of DDT are known to have weak estrogen activity. The organophosphate pesticides fenitrothion, parathion and methylparathion are known to have some androgen and progesterone activity, but no estrogen activity.**

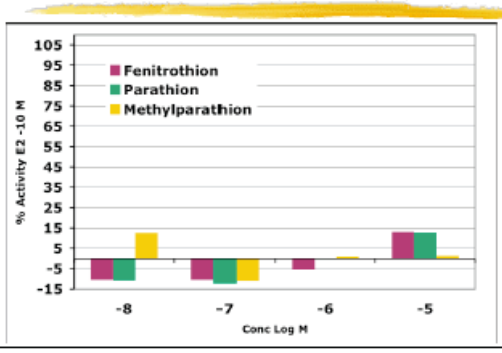
Test Chemicals



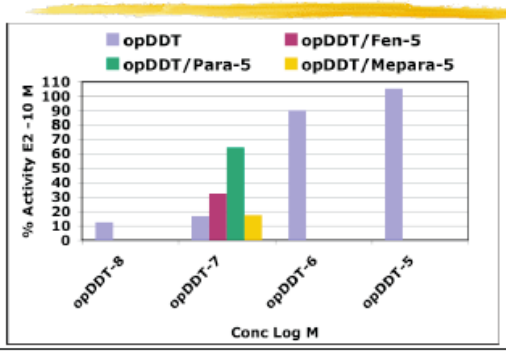
MCF-7 Proliferation: Estradiol-17β



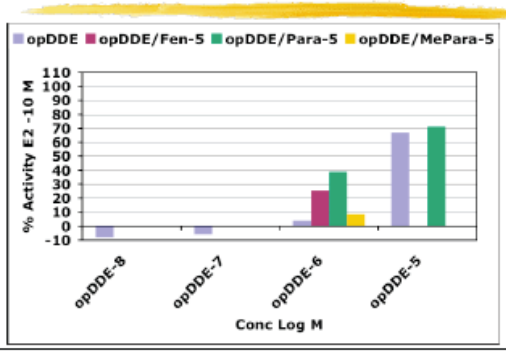
MCF-7 Proliferation: Organophosphate Pesticides



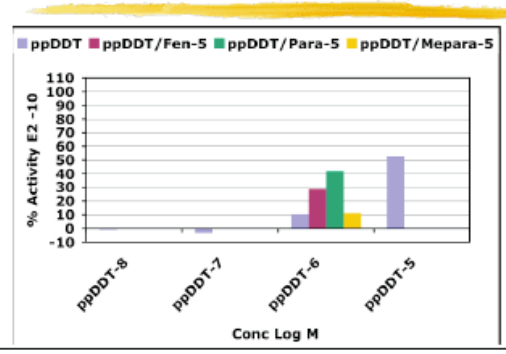
MCF-7 Proliferation: o,p-DDT + Organophosphates



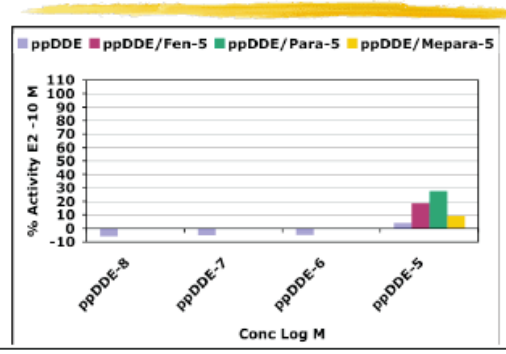
MCF-7 Proliferation: o,p-DDE + Organophosphates



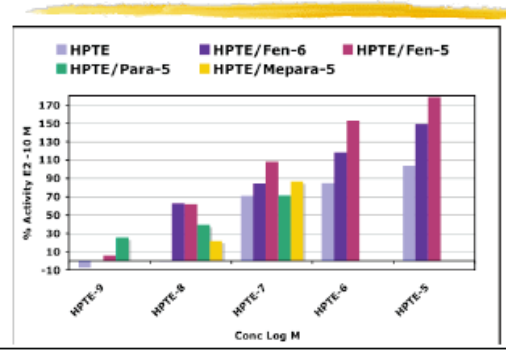
MCF-7 Proliferation: p,p-DDT + Organophosphates



MCF-7 Proliferation: p,p-DDE + Organophosphates



MCF-7 Proliferation: HPTE + Organophosphates



Results and Conclusions

1. **Combining organophosphate pesticides with DDT isomers or metabolites results in greater than additive breast cancer cell proliferation activity.**
2. **Concentrations of DDT isomers or metabolites that alone produce minimal proliferation activity (LOEL) can be sensitized by organophosphate treatment.**
3. **Combinations of pesticides can induce more breast cancer cell proliferation than observed when cells are treated by individual pesticides alone.**

Future Directions

1. **Complete mixture combination matrix (cell proliferation and reporter gene)**
2. **Identify mixtures with greatest proliferative sensitization effect**
3. **Targeted microarray analysis of mixtures (nuclear receptor and breast cancer arrays)**
4. **Identify gene regulation novel to mixtures**

Ongoing and Planned Activities

1. Microarray Experiments at Tulane Cancer Center

- Facility now functional
- Focused experiments: Mixtures of interest
- Use TCC-LCRC Stat Core for analysis

2. Proteomics Analysis at Xavier (Wang Lab)

- Gene product analysis of mixtures
- Develop expertise and collaboration among Xavier cancer researchers

2. Analysis of Chiral Pesticides

- Enantiomer specific estrogen activity

Impact of Hurricane Katrina

Loss due to water damage and power outage:

- Cell lines saved
- $25,000 – Reagents and supplies
- $ 15,000 – Small equipment

Loss in time and research productivity

- 6 months – 12 months
- Lab currently functional

Appendix 2

HBCU/MI Partnership Training Award Program in Breast Cancer at Xavier University of Louisiana and The Tulane Cancer Center

Request for Immediate Assistance for Lab Supplies and One Year of Additional Funding

May 1, 2005

Award Number: W81XWH-04-1-0557

Title:
Developing Breast Cancer Program at Xavier; Genomic and Proteomic Analysis of Signaling Pathways Involved in Xenohormone and MEK5 Regulation of Breast Cancer

Principle Investigator:
Thomas E. Wiese

Contracting organization:
Xavier University of Louisiana
1 Drexel Drive
New Orleans, LA 70125-1098

Contract Period: April 2004 – April 2008

Requested Supplemental Contract Period: April 2008 – April 2009

Background
On August 29th 2005, hurricane Katrina swept just East of New Orleans and then destroyed most of the Mississippi gulf coast. At the same time, this storm inflicted catastrophic damage on the New Orleans area with high winds and then flooding after the failure of multiple flood protection levies. With almost 80% of the city flooded with salt water for most of a month, many homes, business and universities suffered sever damage.

In the aftermath of this storm, it was clear that the HBCU/MI Partnership Training Award at Xavier University of Louisiana and the Tulane Cancer Center (Proposal Number: BC030300) would be impacted. Xavier University of Louisiana suffered damage from 3-6 ft of water on campus flooding all buildings with 1-6 ft of water. The Tulane University Medical Center buildings were also flooded with 1-3 ft of water. Both campuses also lost power and air conditioning for at least 3 months. At the same, about 40% of the faculty and staff at both universities had sever damage to their homes, many a total loss of structure and contents. Fortunately, all the research labs at Xavier and Tulane involved in the DOD HBCU/MI Partnership Training Awards programs were located on 2nd floors or above and were not directly damaged by flood water. The next 4 months after Katrina are now known as the "evacuation" to all of us that went through this experience. While evacuated, most faculty at Xavier and Tulane moved 2-3 times until they settled in a location where they could get involved in research or keep some aspect of their research going. In the case of Dr. Wang and his family, he spent most of his time in Philadelphia and became involved in proteomics work at SFBC Analytical as well as in the Department of Pediatrics at the University of Pensylvania Medical School. Dr. Wiese

became a visiting professor at University of Michigan in the Pharmacology Department. While both of these faculty were able to learn new techniques while evacuated, they also keep in daily contact with their research staff at other locations in the country and then both staff and faculty continued work on projects from their labs at Xavier.

Repair of the Xavier and Tulane Campus

Immediately after the storm and flooding, we all knew that it would be a tough road to bringing back any aspect of the professional and personal lives we knew before Katrina. Lucky for all of us involved in the HBCU/MI Partnership Training Award at Xavier University of Louisiana and the Tulane Cancer Center, our university administrations acted quickly with a sold resolve to bringing back research as well as teaching on both campuses. At Xavier, all the first floors of all buildings on campus were gutted, treated for mold and then remodeled within 4 weeks of the water leaving campus. In the next few months, all electrical, phone and Internet wiring was replaced while the campus physical plant and mechanical rooms in each building were completely rebuilt. Wind damage to various facilities on campus were also repaired. Xavier then replaced lost furniture and fixtures so that repaired areas could be used for the same function as before the storm. When faculty and staff returned to campus on January 9th 2006, there was no sign or smell of water or mold in any building that was remodeled. Only repairs on the original Xavier class room building are lagging behind due to it's designation as a historical landmark. In short, the Xavier campus was fully functional by the January 19 resumption of the 2005 Fall semester. At Tulane Medical Center, repairs moved equally or more rapid. By mid December, faculty were able to come back to work in most laboratories while other parts of the medical center are still being reconstructed in April 2006. The critical aspect of this reconstruction was that both Xavier and Tulane moved quickly to restore not only their teaching facilities, but also their research infrastructure.

Student and Faculty Reductions

While the physical repairs were bing made on both campuses, Xavier and Tulane also went through a restructuring to address the post-Katrina realities of decreased student enrolment and the drastic changes in New Orleans. At Xavier, significant reductions were made in the faculty and staff to reflect the expected enrollment reductions that might reach less than 50% of August 2005. Working under a state of financial emergency, Xavier canceled all contracts and tenure, and then repopulated the university with faculty and staff best suited to achieve the university mission. Xavier is historically black and catholic. The Mission of Xavier is to work towards a more just and humane society. The methods used to carry out this mission are traditional teaching as well as the involvement of students in service and research. Xavier brought back almost all faculty involved in research on campus so Xavier students can stay involved in and keep getting inspired by working on research projects with faculty. Xavier us known for both preparing African American students for medical school as well as graduate programs in the bio-medical sciences. Continuing involvement of students in the DOD cancer training partnerships means that more Xavier pre-med and pre-graduate students are exposed to cancer research and more likely to focus their professional or graduate careers on cancer research. In December 2005, Xavier convened an online re-registration for the fall 2005 semester that would restart in January. This process resulted in almost 80% of all students returning with more than 95% returning in Pharmacy. Xavier then brought back additional faculty and staff so that by January 2006, the total faculty reductions from August 2005 were less than 30%. At Tulane Medical Center, more than 150 faculty were terminated as a result of post-Katrina restructuring. After restructuring and faculty reductions at both Xavier and Tulane, all faculty involved in the DOD HBCU/MI Partnership Training Awards programs (Breast and Prostate) were retained.

Resuming Work at Xavier

When faculty returned to Xavier in January 9th 2006, we were all hopeful and optimistic about bringing back the teaching and research on campus. We were also aware of most challenges. In addition, more than 40% of the faculty and staff at Xavier were dealing with substantial personal stress related to relocation, separation from family and/or the rebuilding of their homes. Work and home presented with extraordinary challenges for most at both universities. At Xavier and the Tulane Medical Center, faculty have been working extra hours to bring back their labs so they can restart their research. This process involved cleaning, inventory and then reordering and restarting each functional capacity of each lab. In general, this process has gone smoothly. Each faculty involved in the DOD HBCU/MI Partnership Training Awards programs at Xavier and Tulane spent the evacuation at a university or cancer center organizing and planning for their return. Xavier's partner in these DOD projects, the Tulane Cancer Center (and the Louisiana Cancer Research Consortium (LCRC)) recognized that all cancer researchers involved with Tulane would need extra financial assistance. The LCRC provided $25,000 grants to cancer researchers involved with the LCRC to assist in the rebuilding and restarting of their laboratories. The Xavier–Tulane DOD Breast Cancer HBCU/MI Partnership program received one of these awards so the Wiese and Wang labs at Xavier, as well as the Burow lab at the Tulane Cancer Center could bounce back as soon as possible. One other stress when returning to work at Xavier has been the fact that due to faculty reductions, many faculty are now teaching extra load or new courses. The Xavier administration is aware of the impact of this issue and has initiated searches for new hires and is also readjusting loads to help the research intensive faculty get back into the lab. In addition, it should be noted that the freshman class that will start at Xavier in September 2006 is expected to be significantly smaller than in August 2005 and it may take a number of years for the Xavier enrolment to return to pre-Katrina levels. During this time, faculty loads will be reduced and new faculty will be hired as the enrolment goes back up.

Post Katrina Realities for this Program: The Need for Additional Funds and Time

While the HBCU/MI Partnership Training Awards programs at Xavier and Tulane survived Katrina with all faculty involved and got a financial boost from the LCRC, the challenges to restarting our cancer research remain significant. While the lost supplies, samples, laboratory animals and research staff have a large impact, it is the lost time that we now recognize as the most debilitating factor in bringing our projects back on line. We have largely replaced critical supplies and repaired and/or restarted damaged facilities and instrumentation and we are now bringing back our research. While this recovery process is going well, it is clear to us now that the time lost from the effects of Katrina is not just the 4-5 months evacuated, but also another 6-7 months it will take to bring our research back to the level it was in August 2005. In addition, the cost of restarting our research is not a simple replacement of lost critical supplies, but also includes the extra supplies needed to restart, revalidate and rebuild our stocks of lost cells cultures and research samples.

Thus, we are now requesting additional funding from the DOD to support the rebuilding and reestablishment of our research during this current recovery year (Program Year 3). In addition, we are asking for a one year funded extension of our HBCU/MI Partnership Training Award program so that we can catch up from this rebuilding year and complete the aims and goals detailed in our plan of work (Year 5).

Additional Supplies for Year 3

Attached below is a program budget for the proposed 5th year of funding. Included in the proposed supplemental budget is $15,000 for lab supplies to assist Drs. Wiese and Wang in restarting and reestablishing their research in the current year. While this project did get lab

supply help from the LCRC, we are still in need of supply funds to bring our projects back to the point they were in August 2005.

Additional Time for the Program

The attached Year 5 budget has been designed with the intent of keeping key operations going for an additional year. Thus, some faculty salary support has been decreased to minimal levels since Drs. Wiese, Hill and Burow have light teaching loads regardless of effort on research. At the same time, the salary support for Dr. Wang, his research staff and the research staff for Dr. Wiese have been maintained at normal levels so that Dr. Wang and the two research staff can dedicate the required time to the research projects. Recognizing that this program allows carry over of funds, we have reduced the supply budget to minimal levels for all projects in year 5. We expect to carry over significant funds into year 5 that can be used for supplies. We also have dropped funding for research focus groups and our yearly symposia in year 5. Our group of cancer researchers has been interacting well with the Tulane cancer Center, we participate in their meetings and symposia and so eliminating meetings derived from the DOD cancer training projects in year 5 should not have an effect on the over all goals of the program.

Detailed Cost Estimate Form

Name of Principal Investigator *(last, first, middle)* Wiese, Thomas

DETAILED BUDGET - XOD BREAST CANCER YEAR 5					**FROM 05.01.08**		**THROUGH 04.30.09**
PERSONNEL		**TYPE APPT. (MONTHS)**	**ANNUAL BASE SALARY**	**% EFFORT ON PROJECT**	**DOLLAR AMOUNT REQUESTED (OMIT CENTS)**		
NAME	**ROLE ON PROJECT**				**SALARY REQUESTED**	**FRINGE BENEFITS**	**TOTALS**
Wiese, Thomas	**Principal Investigator**	12	84,978	10	8,498	1,360	9,858
Steven Hill	Alternate PI & Mentor	12	143,699	5	7,185	1,078	8,335
Matthew Burow	Mentor	12	106,430	5	5,322	798	6,120
Guangdi Wang	Researcher	9	59,057	20	11,811	1,890	13,701
Research Staff for Dr. Wiese	Post Doc	12	32,612	100	32,612	5,218	37,830
XU Junior Faculty	Researcher	12	40,000	50	20,000	3,200	23,200
Research Staff for Dr. Wang	Post Doc	12	37,032	100	37,032	5,925	42,957
Research Specialist for Dr. Burow	Medical Res. Specialist	12	49,970	40	19,988	2,998	22,986
Program Assistant	Admin.	12	41,305	25	10,326	1,652	11,978
SUBTOTALS →→→→→					$152,774	$24,119	**$176,893**
MAJOR EQUIPMENT (ITEMIZE).							0
MATERIALS, SUPPLIES, AND CONSUMABLES (ITEMIZE BY CATEGORY)							34,000
TRAVEL COSTS							7,200
OTHER EXPENSES (ITEMIZE BY CATEGORY)							6,000
SUBTOTAL OTHER DIRECT COSTS FOR INITIAL BUDGET PERIOD →→→→→							$47,200
SUBCONTRACT COSTS	**DIRECT COST**						
	INDIRECT COST						
TOTAL PERSONNEL AND OTHER DIRECT COSTS FOR INITIAL BUDGET PERIOD							$224,093
TOTAL INDIRECT COSTS FOR INITIAL BUDGET PERIOD							$83,944
TOTAL COSTS FOR INITIAL BUDGET PERIOD							$308,037

JUSTIFICATION: FOLLOW THE BUDGET JUSTIFICATION INSTRUCTIONS EXACTLY. USE CONTINUATION PAGES AS NEEDED.

a. Personnel

FACULTY LABOR Total = $52816.

Thomas Wiese/PI - $8498 = 10% release time from a 12-month appointment to carry out activities as PI. Dr. Wiese will be responsible for coordinating workshops, reviewing the preparation of annual progress reports to the DoD and reports to the Tulane-Xavier Partnership Committee, and overseeing the budget and use of funds, preparing and coordinating supplemental applications to sustain the Breast Cancer Program, approving purchase orders, paperwork and budget calculations for the fiscal office, and encouraging faculty participation and development. The PI will be responsible for the junior faculty budget and activities.
Steven Hill/CoPI-Alternate PI/Mentor - $7185=5% release time from a 12 month appointment is requested. Dr. Hill will provide guidance to Dr. Wiese in the preparation of the reports and organizing the workshops. Dr. Hill will serve as a mentor to Dr. Wiese in his project.
Guangdi Wang/ Researcher - $11,811=20% release time from a 9-month appointment during the school year is requested. Dr. Wang will be responsible for the training of the lab technician and student, supervision of the experiments and communication with the mentor – Dr. Burow. Dr. Wang will also administer the budget for his project and prepare manuscripts.
Matthew Burow/Mentor - $5,322=5% release time from a 12 month appointment is requested. Dr. Burow will serve as a mentor to Dr. Wang in his project.
Junior Faculty - $20,000=50% release time from a 12-month appointment for a Junior Faculty is requested. The junior faculty will participate in seminars and workshops.

STAFF LABOR Total = $99,958

Medical Research Specialists/Dr. Burow - $19,988=40% effort on the project is requested. The specialist will be responsible for the performance of the experiments suggested in Dr. Burow's project.
Research Staff/Dr, Wang - $37,032=100% effort on the project is requested. The Post-Doc will be responsible for the performance of the experiments suggested in Dr. Wang's project. The PostDoc will also train the XU undergraduate student.
Research Staff/Dr. Wiese - $32,612=100% effort on the project is requested. Salary support (12 month) is requested for Mr. Chris Segar, currently a research assistant in the Wiese lab. He will dedicate 100% of his time to the support this project with cell culture tasks involved with proliferation assays and gene array RNA preps, making of primers, running of Northern and Western blots on cell preparations as well as rtPCR analysis in the Hill Lab at Tulane. He will also interface with the Tulane Cancer Center Gene Therapy unit to run micro array analysis of treated breast cancer cells.
Grants Manager/Program Assistant for PI - $10,326=25% effort on the project is requested. Ms. Stephanie Colbert will assist Dr. Wiese with grant management as well as planning meetings, workshops and coordinating general communication activities in this program project. Xavier will wave the indirect costs on the salary for this position.

b. Fringe Benefits: Total of $24,119
TCC Faculty – Faculty wages have 15% Fringe benefit that adds up to $1876. Please see attached rate agreement.

TCC Research Staff – Research staff wages have 15% Fringe benefit that adds up to $2998. Please see attached rate agreement.
XU Faculty and Research Staff – 16% Fringe benefits are calculated for all XU faculty and Post-Docs for the sum of $19,245.

c. Major Equipment: No major equipment is requested for this period.

d. Materials, Supplies, and Consumables: Total: $34,000
The costs of chemicals and supplies are distributed as follows. Dr. Wang's lab ($5,000) will purchase the chemicals needed for the proteomic project. Dr. Burow's lab ($4,000) will purchase the cells and cell culture media and disposable to provide Dr. Wang with the biological samples for proteomic analysis. Dr. Wiese ($10,000) will purchase the tissue culture chemicals and disposables, antibodies, and other chemicals needed. In addition, $15,000 is requested for immediate use by Drs. Wang and Wiese to bring their projects back to pre-Katrina status.

e. Travel Costs: Total = $7,200.
TCC Travel – Dr. Hill has $1,800 to use for conferences in Breast Cancer.
XU Researchers Travel – Drs. Wang and Wiese are each allocated $1,800 for travel to annual cancer symposia to present their research.
Junior Faculty Travel – Junior Faculty is allocated $1,800 for travel to annual cancer symposia to present their research.

g. Other Expenses: Total: $6,000
Maintenance and Repairs - The sum of $6000 is requested to support the maintenance and repair of equipment in Drs. Wiese and Wang's labs.

i. Indirect Costs (overhead, general and administrative, and other): Total: $83,944.
TCC Indirect Cost – 48% are calculated for the entire TCC budget (Dr. Hill and Dr. Burow). The total indirect cost for the TCC is $20,721. Please see attached rate agreement.

XU Indirect Cost – 57.5% are calculated for the wages of all XU personnel including faculty, research staff, and students. The total indirect cost for XU is $63,223. Please see attached rate agreement.

Appendix 3

Department of Defense
Congressionally Directed Medical Research Programs at
Xavier University of Louisiana

Breast Cancer and Prostate Cancer Research
Noon – 1:00pm
Monthly Research Meeting Schedule

Date: Presenters

Monday September 7, 2006 Agenda: Status of Xavier DOD HBCU/MI Prostate Cancer and Breast Cancer Training
Attendees: David Wolfgang, Charles Miller, Guandi Wang, Matt Burow, Thomas Wiese, Asim Abdel-Mageed, Suresh Sikka, Steven Hill, Shubha Ireland, Cheryl Stevens, Dr. Shankar, Stephanie Colbert

Monday October 16, 2006 Wiese-Hill Project
Attendees: David Wolfgang, Guandi Wang, Shubha Ireland, Matt Burow, Thomas Wiese, Asim Abdel-Mageed, Steven Hill, Cheryl Stevens, Dr. Shankar, Chris Segar, W. Ming, Maryam Foroozesh, Stephanie Colbert

Monday November 6, 2006 Wang-Burow Project
Attendees: David Wolfgang, Guandi Wang, Matt Burow, Thomas Wiese, Asim Abdel-Mageed, Steven Hill, Cheryl Stevens, Dr. Shankar, Chris Segar, W. Ming, Maryam Foroozesh, Stephanie Colbert

Monday December 4, 2006 Steven-Jones Project
Attendees: David Wolfgang, Guandi Wang, Thomas Wiese, Frank Jones, Cheryl Stevens, Dr. Shankar, Chris Segar, W. Ming, Maryam Foroozesh, Stephanie Colbert, Dr. Wang's lab tech

Monday February 5, 2007 Wiese-Hill Project
Attendees: Suresh Sikka, Steven Hill, Abdel Mageed, Shubha Ireland, Chris Segar, Kirk Williams, Cheryl Stevens, Maryam Foroozesh, Gurandi Wang, David Wolfgang, 3 lab techs, Thomas Wiese, Stephanie Colbert

Monday March 26, 2007 Ireland-Mageed Project
Attendees: Suresh Sikka, Steven Hill, Abdel Mageed, Shubha Ireland, Chris Segar, Kirk Williams, Michael ? (student), David Wolfgang, 3 lab techs, Thomas Wiese, Stephanie Colbert

Monday April 30, 2007 Wolfgang-Miller Project
Attendees: David Wolfgang, Charles Miller, Thomas Wiese, Cheryl Stevens, Dr. D. Johnson, Chris Segar, W. Ming, Maryam Foroozesh, Stephanie Colbert, Dr. Wang's lab tech